1975

The Chemistry and Biochemical Systematics of the Sesquiterpene Lactones in Melampodium (Compositae).

Donald Lee Perry
Louisiana State University and Agricultural & Mechanical College

THE CHEMISTRY AND BIOCHEMICAL SYSTEMATICS OF THE SESQUITERPENE LACTONES IN MELAMPODIUM (COMPOSITAE)

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy

in

The Department of Chemistry

by

Donald Lee Perry

B.S., University of Wisconsin-Madison, 1968

May, 1975

To Mom and Dad

A Dream Fulfilled

ACKNOWLEDGEMENT

I wish to acknowledge the firm guidance, personal inspiration, and friendship provided by my major professor, Nikolaus H. Fischer. Particular gratitude is felt for the patience shown and the personal freedom given by Dr. Fischer to me during the course of my graduate study. I also have the special honor of being Dr. Fischer's first graduating Ph.D. student.

The collection and identification of the various plants by Dr. Tod Stuessy is greatly appreciated. Special thanks go to Joe and Judy Abraham for their technical assistance in the extraction of the many plant populations. The numerous discussions with Dr. George R. Newkome are remembered for their valuable contributions to my understanding of Organic Chemistry.

I gratefully acknowledge the financial support received from the Dr. Charles E. Coates Memorial Fund of the Louisiana State University Foundation donated by George H. Coates for the preparation of this dissertation.

Donald Lee Perry

Baton Rouge, Louisiana

April, 1975

TABLE OF CONTENTS

TABLE OF CONTENTS CONTINUED PAGE

LIST OF TABLES

TABLES CONTINUED PAGE

LIST OF FIGURES

LIST OF SCHEMES

SCHEME PAGE

SCHEME PAGE

ABSTRACT

During the course of a biochemical systematic study of the genus Melampodium (Compositae:Heliantheae) several new sesquiterpene lactones were isolated and characterized by spectroscopic methods and chemical correlations. The germacranolide dilactones, melampodin-C (64), cinerenin (67), and 4(5)-dihydromelampodin-B (61) were isolated from white-rayed species, while the melampolide melampodinin (70) represents the first sesquiterpene lactone found in a member of the yellow-rayed complex of Melampodium. The incomplete structure determination of a melampodin-A like melampolide, which was isolated from M. cinereum, is also included. Two known compounds, melampodin-B (56) and the flavone artemetin (78), were also present in most populations of M. cinereum and M. argophyllum.

The structures of cinerenin ($C_{17}H_{20}O_6$), 4(5)-dihydro-melampodin-B ($C_{17}H_{20}O_7$), and melampodin-C ($C_{19}H_{22}O_7$) were elucidated mainly by correlating the nmr parameters and mass spectral fragmentation patterns with those of melampodin-B. The mass spectral peaks at m/e 274, 256, and 228 were found to be typical of the germacranolide dilactones which contain a medium ring similar to that of melampodin-B.

The conversion of melampodin-B, cinerenin, melampodin-C, and 4(5)-dihydromelampodin-B to the respective acetates, aldehydes, and/or dihydro-derivatives, corroborated their structural and nmr spectral assignments as well as their empirical formulas. The

ABSTRACT

During the course of a biochemical systematic study of the genus Melampodium (Compositae:Heliantheae) several new sesquiterpene lactones were isolated and characterized by spectroscopic methods and chemical correlations. The germacranolide dilactones, melampodin-C (64), cinerenin (67), and 4(5)-dihydromelampodin-B (61) were isolated from white-rayed species, while the melampolide melampodinin (70) represents the first sesquiterpene lactone found in a member of the yellow-rayed complex of Melampodium. The incomplete structure determination of a melampodin-A like melampolide, which was isolated from M. cinereum, is also included. Two known compounds, melampodin-B (56) and the flavone artemetin (78), were also present in most populations of M. cinereum and M. argophyllum.

The structures of cinerenin ($C_{17}H_{20}O_6$), 4(5)-dihydro-melampodin-B ($C_{17}H_{20}O_7$), and melampodin-C ($C_{19}H_{22}O_7$) were elucidated mainly by correlating the nmr parameters and mass spectral fragmentation patterns with those of melampodin-B. The mass spectral peaks at m/e 274, 256, and 228 were found to be typical of the germacranolide dilactones which contain a medium ring similar to that of melampodin-B.

The conversion of melampodin-B, cinerenin, melampodin-C, and 4(5)-dihydromelampodin-B to the respective acetates, aldehydes, and/or dihydro-derivatives, corroborated their structural and nmr spectral assignments as well as their empirical formulas. The

conversion of melampodin-B, cinerenin, and melampodin-C to the tribromide 60 provided chemical evidence that the cyclodeca-1,5-diene ring portions of these compounds are identical.

The structure of melampodinin ($C_{25}H_{30}O_{12}$), a melampolide isolated from M. americanum, was determined by the use of ir and pmr spectroscopy. The spectra of melampodinin and of melampodin-A acetate, a structure of known absolute configuration, are almost identical. The stereochemistries of melampodin-B, melampodin-C, and cinerenin were derived by correlating the respective coupling constants of the H-1, H-5, H-6, H-7, H-8, and H-9 signals with stereomodel considerations and the following biogenetic assumptions: 1) the H-7 is α-oriented, and 2) the C-4(5) double bond has a trans configuration. The correlation of the allylic coupling value between the respective H-1 and H-9 signals with the observed torsional angle of these protons substantiated the assignment of a β-oriented substituent at C-1 in these compounds and their derivatives.

Using the aforementioned sesquiterpene lactones as biogenetic markers in the biochemical systematic study of about 50 populations of M. cinereum and M. argophyllum the following conclusion is drawn: M. argophyllum is phylogenetically more closely related to M. cinereum than it is to M. leucanthum. This conclusion, which is contrary to Stuessy's morphological considerations, is based on the fact that the sesquiterpene lactones in M. argophyllum and M. cinereum are distinctly different from those in M. leucanthum.

conversion of melampodin-B, cinerenin, and melampodin-C to the tribromide 60 provided chemical evidence that the cyclodeca-1,5-diene ring portions of these compounds are identical.

The structure of melampodinin ($C_{25}H_{30}O_{12}$), a melampolide isolated from M. americanum, was determined by the use of ir and pmr spectroscopy. The spectra of melampodinin and of melampodin-A acetate, a structure of known absolute configuration, are almost identical. The stereochemistries of melampodin-B, melampodin-C, and cinerenin were derived by correlating the respective coupling constants of the H-1, H-5, H-6, H-7, H-8, and H-9 signals with stereomodel considerations and the following biogenetic assumptions: 1) the H-7 is α-oriented, and 2) the C-4(5) double bond has a trans configuration. The correlation of the allylic coupling value between the respective H-1 and H-9 signals with the observed torsional angle of these protons substantiated the assignment of a β-oriented substituent at C-1 in these compounds and their derivatives.

Using the aforementioned sesquiterpene lactones as biogenetic markers in the biochemical systematic study of about 50 populations of M. cinereum and M. argophyllum the following conclusion is drawn: M. argophyllum is phylogenetically more closely related to M. cinereum than it is to M. leucanthum. This conclusion, which is contrary to Stuessy's morphological considerations, is based on the fact that the sesquiterpene lactones in M. argophyllum and M. cinereum are distinctly different from those in M. leucanthum.

INTRODUCTION

Plants produce a wide variety of different types of natural products. Many secondary plant metabolites* are wide-spread in the plant Kingdom while others are restricted to specific plant families, tribes, genera, and species. Different populations of one species frequently produce distinctly different structural types within a class of natural products.

The plant family Compositae (the sunflower family) is known to produce many different structural types of sesquiterpene lactones. Furthermore, different tribes, genera, and species of this family rather specifically contain certain types of sesquiterpene lactones which generally have in common the typical α, β-unsaturated γ-lactone structure (structure 1). During the last decade, sesquiterpene lactones have attracted considerable attention for two major reasons: they are commonly used as biogenetic markers in biochemical systematic studies,[2-5] and secondly, in the search for anti-tumor active natural products a number of different types of sesquiterpene lactones have shown promising activity.[6,7]

The primary reason for the use of sesquiterpene lactones in biochemical systematic studies is the existence of a variety of

---

*The term "natural product" is recognized by the chemist as meaning those secondary metabolites, usually of relatively complex structure, which are of more restricted distribution and more characteristic of specific bontanical sources than are the compounds produced by primary metabolic processes.

INTRODUCTION

Plants produce a wide variety of different types of natural products. Many secondary plant metabolites* are wide-spread in the plant Kingdom while others are restricted to specific plant families, tribes, genera, and species. Different populations of one species frequently produce distinctly different structural types within a class of natural products.

The plant family Compositae (the sunflower family) is known to produce many different structural types of sesquiterpene lactones. Furthermore, different tribes, genera, and species of this family rather specifically contain certain types of sesquiterpene lactones which generally have in common the typical α, β-unsaturated γ-lactone structure (structure 1). During the last decade, sesquiterpene lactones have attracted considerable attention for two major reasons: they are commonly used as biogenetic markers in biochemical systematic studies,[2-5] and secondly, in the search for anti-tumor active natural products a number of different types of sesquiterpene lactones have shown promising activity.[6,7]

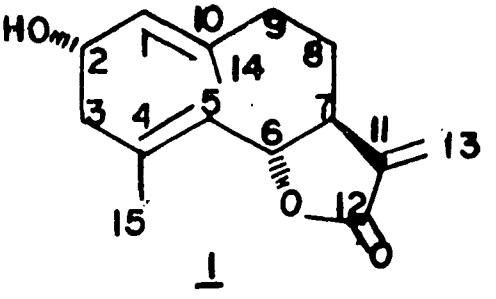
The primary reason for the use of sesquiterpene lactones in biochemical systematic studies is the existence of a variety of

---

*The term "natural product" is recognized by the chemist as meaning those secondary metabolites, usually of relatively complex structure, which are of more restricted distribution and more characteristic of specific bontanical sources than are the compounds produced by primary metabolic processes.

structural types as well as their restricted occurrence in specific taxa. In the following, a typical example of each structural class will be shown:

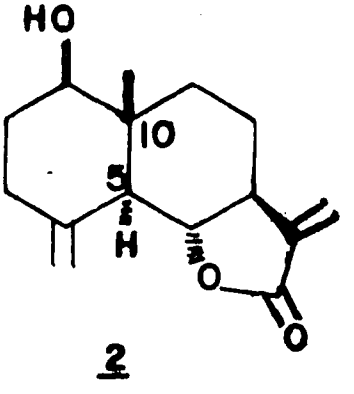
1. The germacranolides represent cyclodeca-1,5-dienes which usually contain trans,trans double bonds at the C-4(5) and C-1(10) positions of the medium ring. Tamaulipin-A(1)[8] is a typical example.



1

TAMAULIPIN-A

2. The eudesmanolides are typically represented by reynosin (2)[9] and are characterized by a 6,6-fused ring system; they normally have a trans-decaline skeleton.



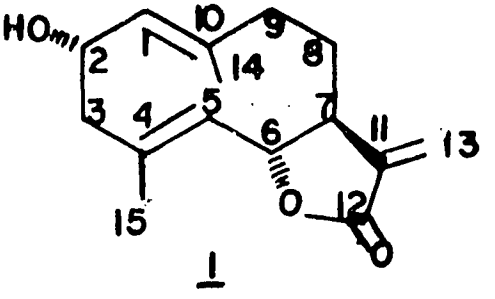
2

REYNOSIN

3. The eremophilenolides differ from the eudesmanolides by the position of a methyl group; that is, the presence of a methyl group at C-5 instead of C-10. A typical example is eremophilenolide (3).[10]

structural types as well as their restricted occurrence in specific taxa. In the following, a typical example of each structural class will be shown:

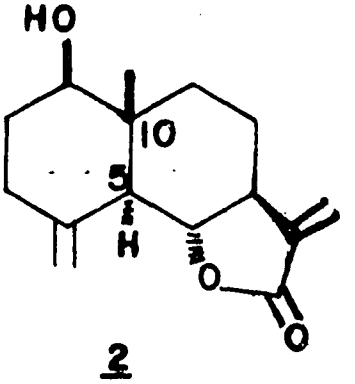
1. The germacranolides represent cyclodeca-1,5-dienes which usually contain trans,trans double bonds at the C-4(5) and C-1(10) positions of the medium ring. Tamaulipin-A(1)[8] is a typical example.



1

TAMAULIPIN-A

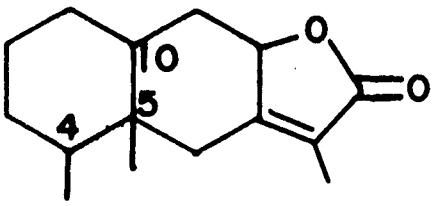
2. The eudesmanolides are typically represented by reynosin (2)[9] and are characterized by a 6,6-fused ring system; they normally have a trans-decaline skeleton.



2

REYNOSIN

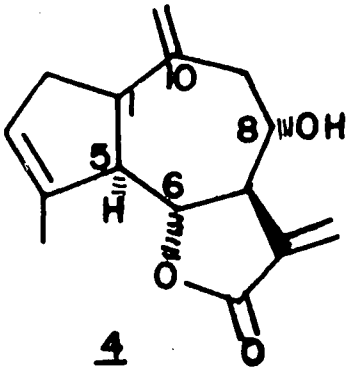
3. The eremophilenolides differ from the eudesmanolides by the position of a methyl group; that is, the presence of a methyl group at C-5 instead of C-10. A typical example is eremophilenolide (3).[10]



EREMOPHILENOLIDE

3

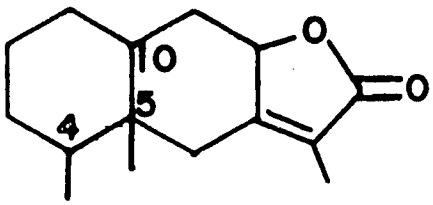
4. The guaianolides have a 5,7 ring system and are based upon the guaiane skeleton. Rupicolin-B (4)[11] is a representative of this class.



RUPICOLIN—B

4

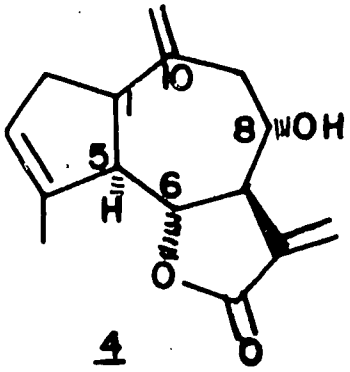
5. The pseudoguaianolides are the largest class of sesquiterpene lactones and they also represent derivatives of a 5,7-ring system which, in contrast to the guaianolides, contain a methyl group at the C-5 instead of the C-4 position. They commonly occur as γ-lactones and are lactonized to either the C-6 or C-8 positions in the seven-membered ring. They may also be cleaved between the C-3 and C-4 or the C-4 and C-5 positions. Confertiflorin (5)[12] is an example of a γ-lactone with a cis-ring closure to C-6.



EREMOPHILENOLIDE

3

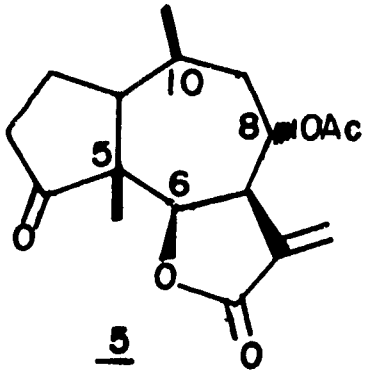
4. The guaianolides have a 5,7 ring system and are based upon the guaiane skeleton. Rupicolin-B (4)[11] is a representative of this class.



RUPICOLIN—B

4

5. The pseudoguaianolides are the largest class of sesquiterpene lactones and they also represent derivatives of a 5,7-ring system which, in contrast to the guaianolides, contain a methyl group at the C-5 instead of the C-4 position. They commonly occur as γ-lactones and are lactonized to either the C-6 or C-8 positions in the seven-membered ring. They may also be cleaved between the C-3 and C-4 or the C-4 and C-5 positions. Confertiflorin (5)[12] is an example of a γ-lactone with a cis-ring closure to C-6.

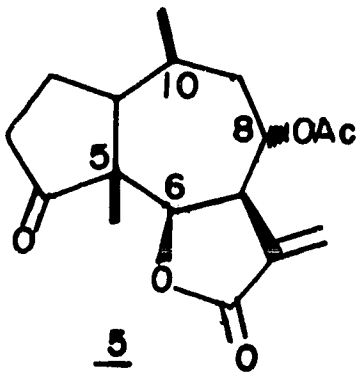


5

CONFERTIFLORIN

The understanding of the biosynthesis of sesquiterpene lactones is still in the speculative stage due to the extreme difficulties in the administration techniques of appropriate labeled precursors to the plants. In most experiments very low incorporation rates are observed when either live plants or cell cultures are used. Despite these limitations, the biosynthetic pathways leading to sesquiterpene lactones are believed to be fairly well understood. The recognition of the central role of mevalonic acid (6) in the biosynthesis of monoterpenes and steroidal compounds[13-15] has led to generalizations of these known pathways to other terpenoids which, with the appropriate caution, can be helpful in structural and systematic considerations. The following general scheme outlines a hypothetical pathway of sesquiterpenoid biosynthesis (Scheme I).

The transformation of farnesyl pyrophosphate (7) into the sesquiterpene lactone end products lacks detailed and conclusive experimental evidence for their total biosynthetic pathways. Scanty evidence,[16-19] however, together with the compelling nature of the biosynthetic hypothesis are pervasive enough for the following route to be generally accepted:



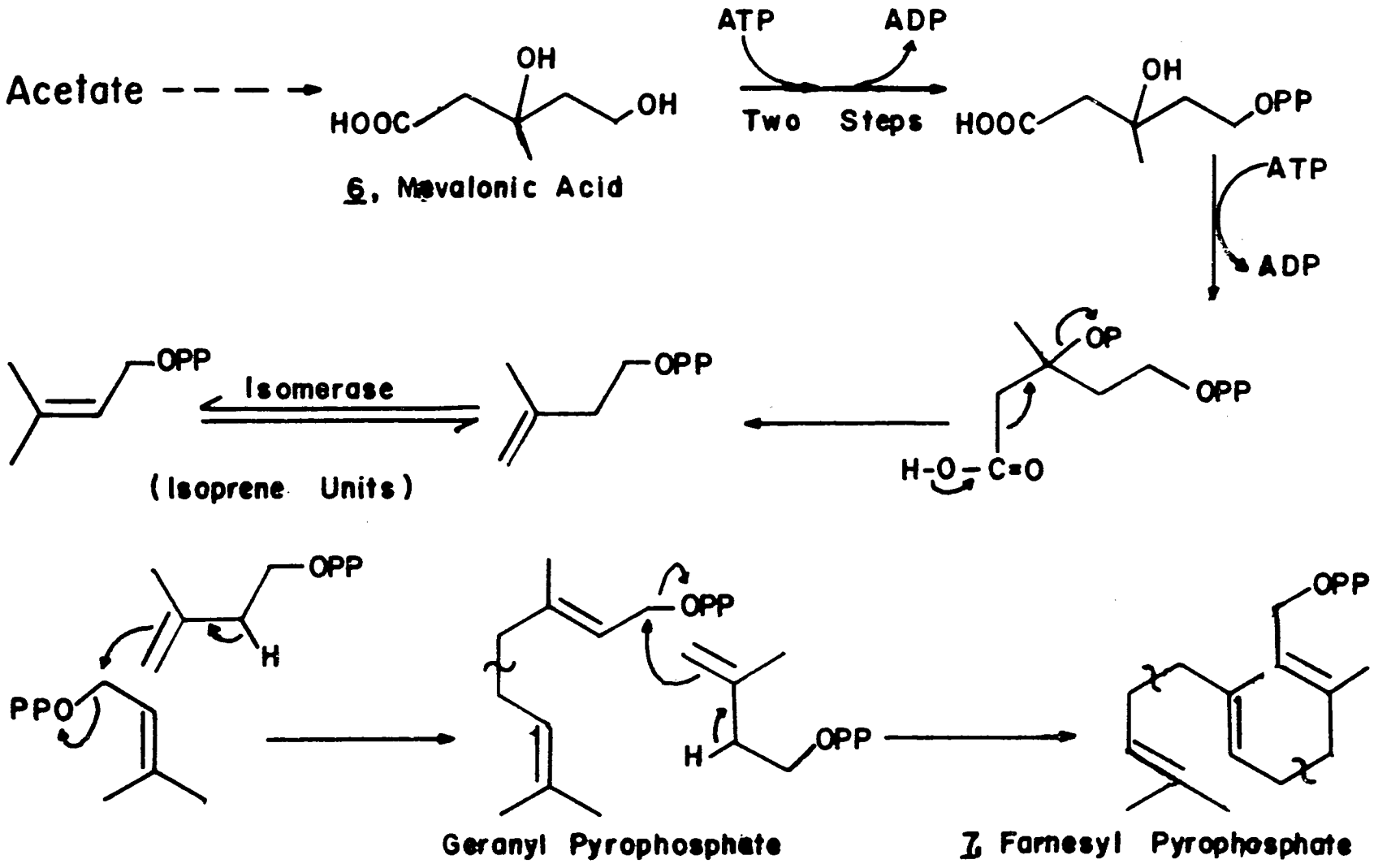
5

CONFERTIFLORIN

The understanding of the biosynthesis of sesquiterpene lactones is still in the speculative stage due to the extreme difficulties in the administration techniques of appropriate labeled precursors to the plants. In most experiments very low incorporation rates are observed when either live plants or cell cultures are used. Despite these limitations, the biosynthetic pathways leading to sesquiterpene lactones are believed to be fairly well understood. The recognition of the central role of mevalonic acid (6) in the biosynthesis of monoterpenes and steroidal compounds[13-15] has led to generalizations of these known pathways to other terpenoids which, with the appropriate caution, can be helpful in structural and systematic considerations. The following general scheme outlines a hypothetical pathway of sesquiterpenoid biosynthesis (Scheme I).

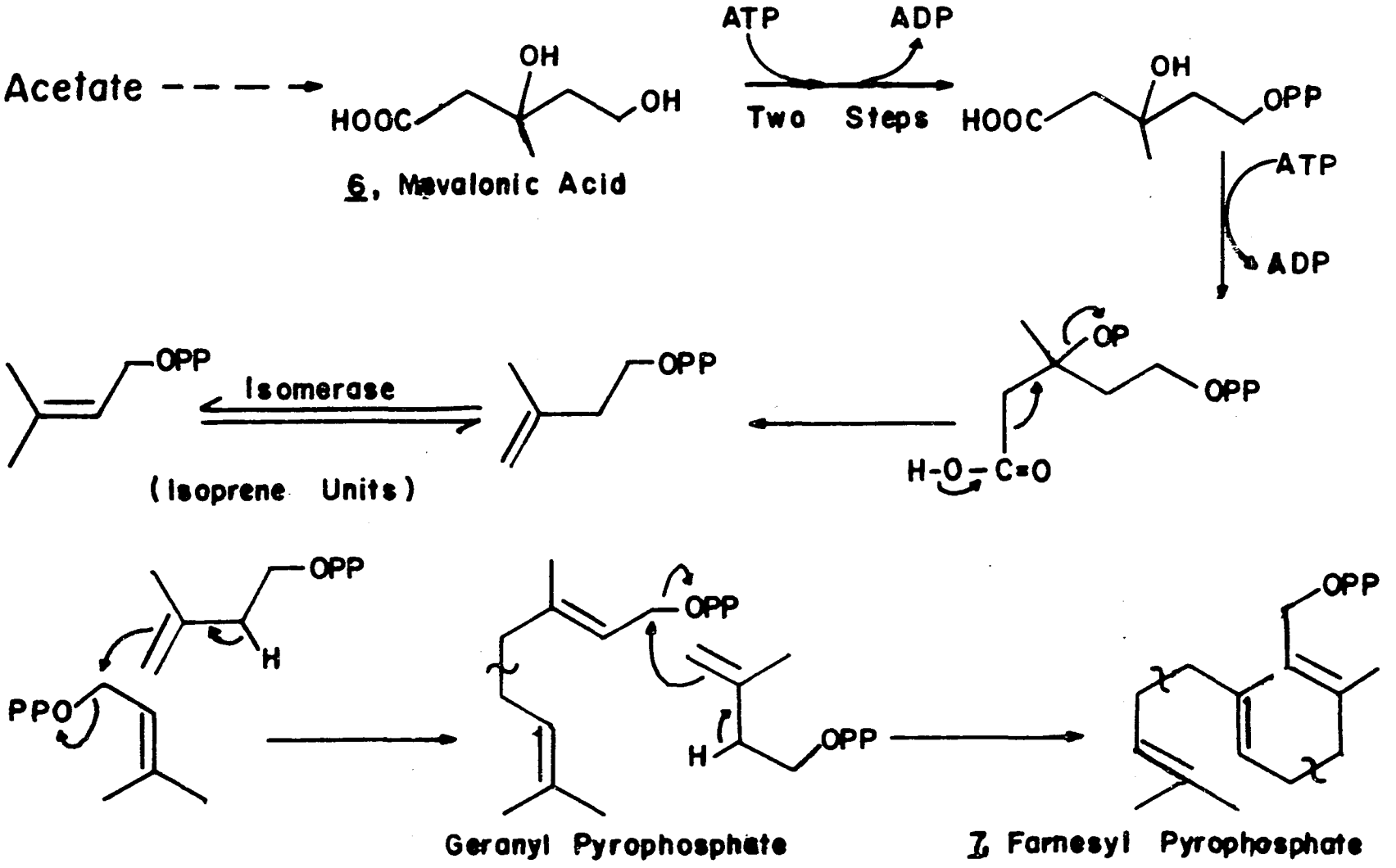
The transformation of farnesyl pyrophosphate (7) into the sesquiterpene lactone end products lacks detailed and conclusive experimental evidence for their total biosynthetic pathways. Scanty evidence,[16-19] however, together with the compelling nature of the biosynthetic hypothesis are pervasive enough for the following route to be generally accepted:

Probable Biosynthetic Pathway To The Sesquiterpenoids



SCHEME I

Probable Biosynthetic Pathway To The Sesquiterpenoids

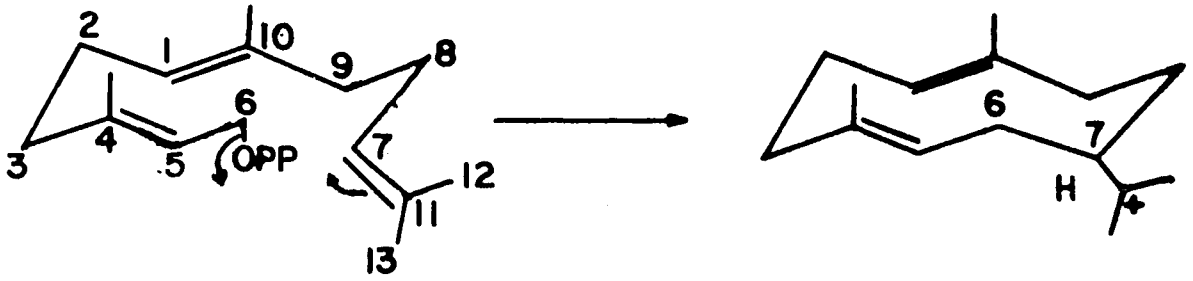


6, Mevalonic Acid

Geranyl Pyrophosphate

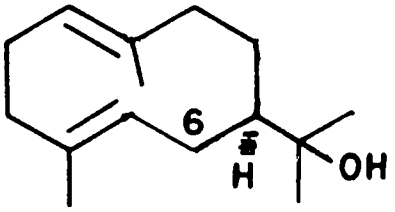
7, Farnesyl Pyrophosphate

SCHEME I

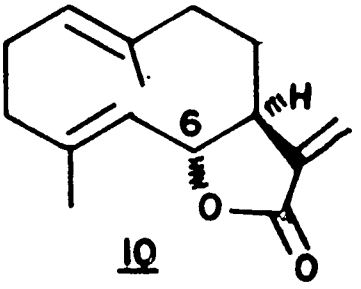


7, trans,trans Farnesyl pyrophosphate 8

Hedycarol (9) is the simplest monocyclic sesquiterpene derived from 8. The formation of costunolide (10) from 8 requires two changes:

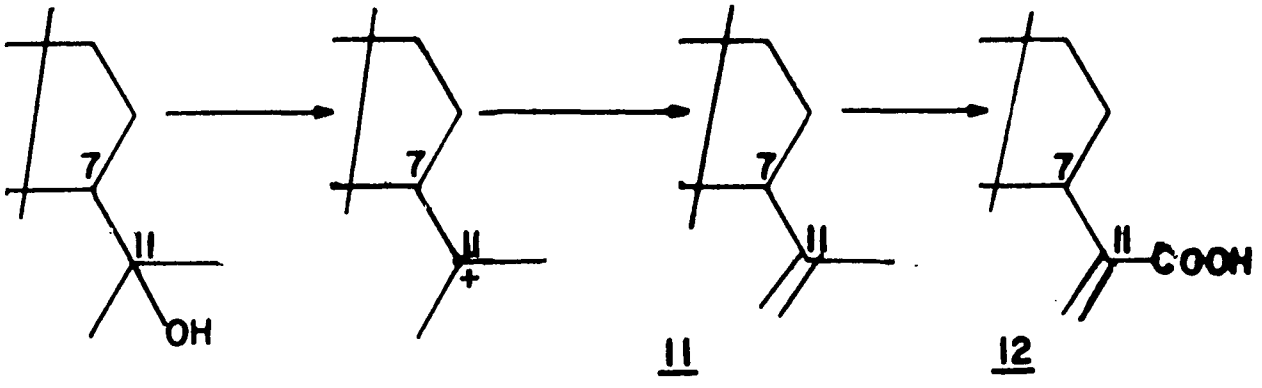


9



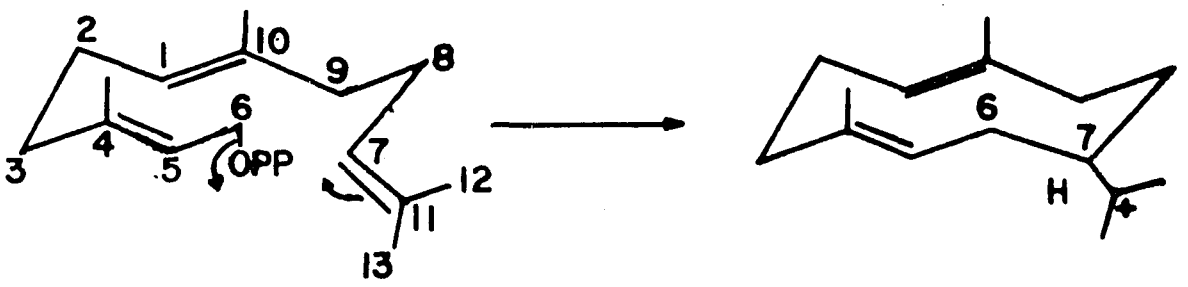
10

(a) the introduction of oxygen into position 6, and (b) the oxidative transformation of the isopropyl group, possibly in the following way (Scheme II):



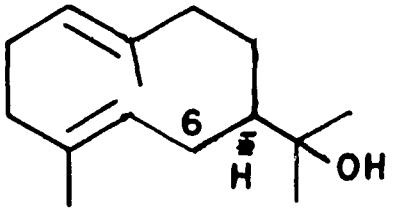
11 12

SCHEME II

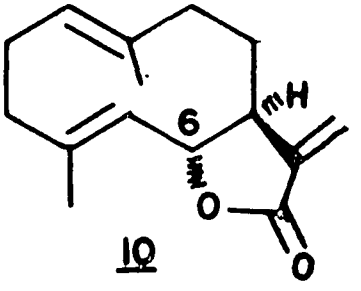


7, trans,trans Farnesyl pyrophosphate 8

Hedycarol (9) is the simplest monocyclic sesquiterpene derived from 8. The formation of costunolide (10) from 8 requires two changes:

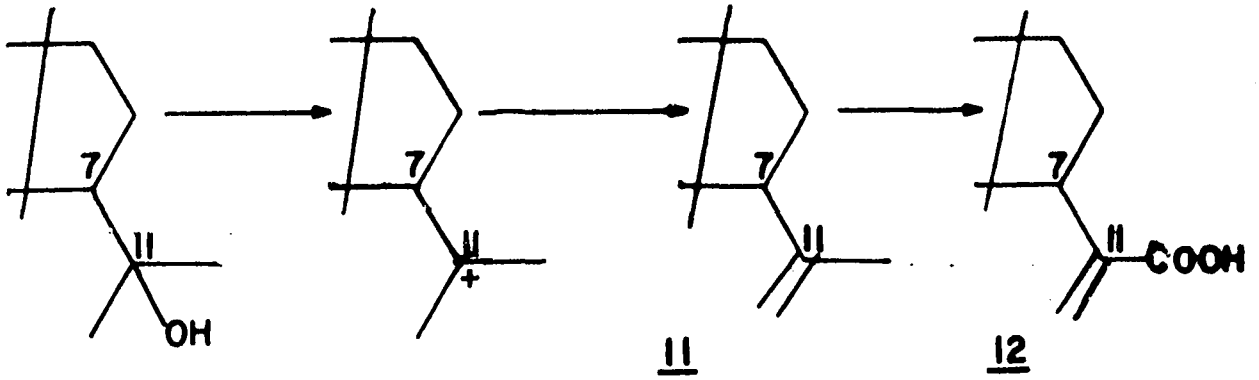


9



10

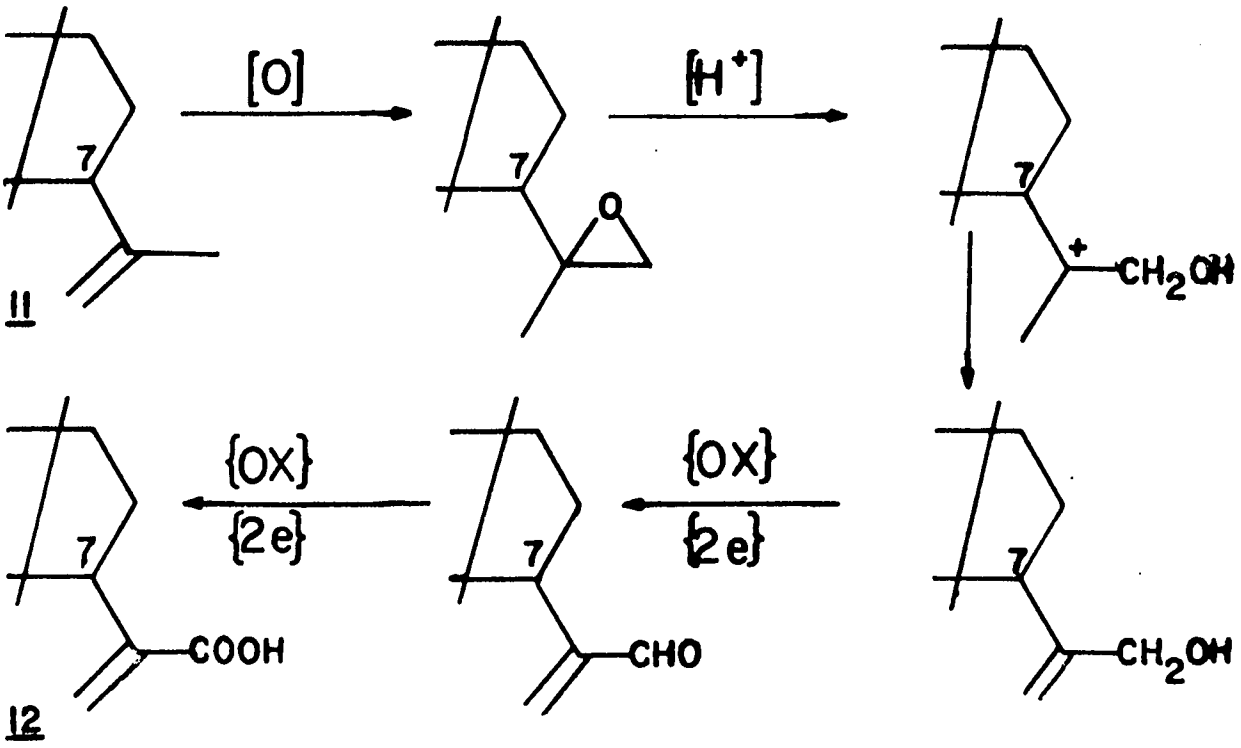
(a) the introduction of oxygen into position 6, and (b) the oxidative transformation of the isopropyl group, possibly in the following way (Scheme II):



11 12

SCHEME II

Details about the biomodifications leading to the α,β-unsaturated γ-lactones in plants are still unknown. Expoxidation, however, is believed to be the initial step in many biomodifications such as the transformation of squalene to steroidal compounds *via* squalene 2,3 oxide.[20] The large number of naturally occurring epoxide terpenoids also gives credence to this assumption. A reasonable pathway suggested by Geissman is illustrated in Scheme III.[21]

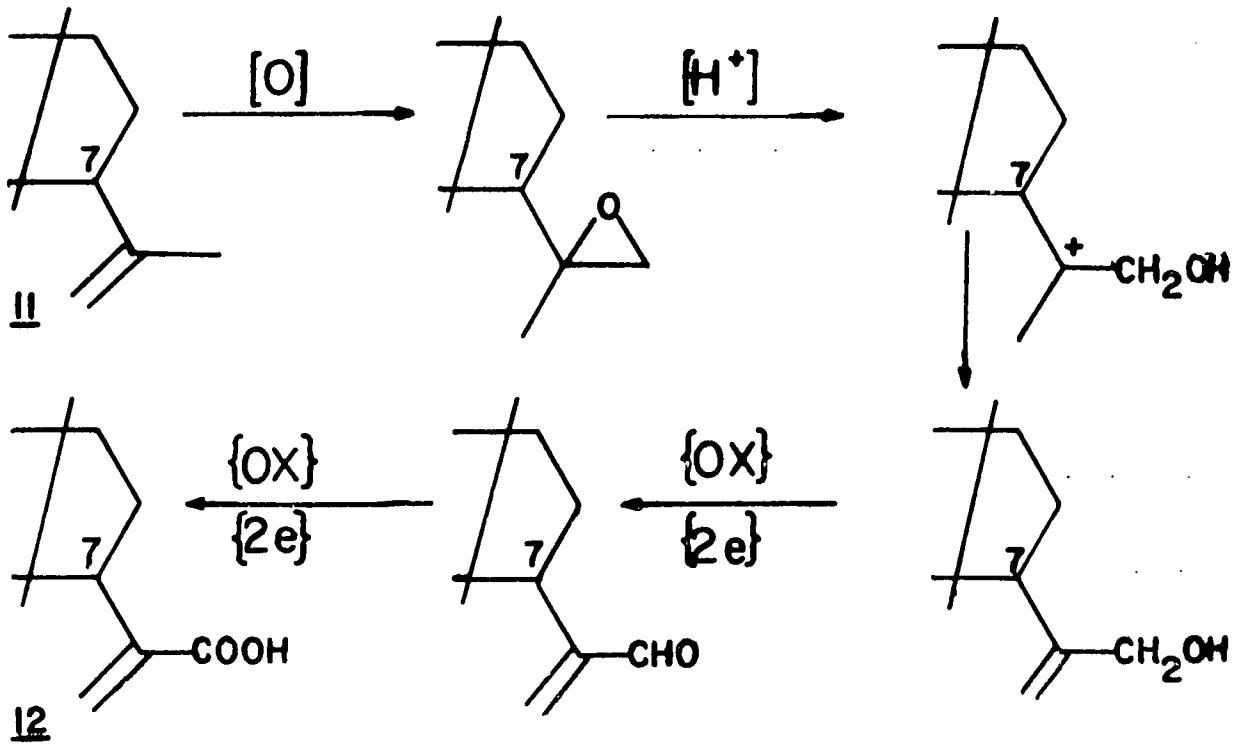


SCHEME III

In fact, trans-annular ring closure of the cyclodecadiene germacranolides to give the different structural types of bicyclic terpenoids is believed to proceed also *via* epoxide intermediates.[22]

The formation of guaianolides and pseudoguaianolides from 13 is realized by stereospecific cyclizations leading to the guaiane and pseudoguaiane skeletons. Pseudoguaianolides (14) may be formed

Details about the biomodifications leading to the α,β-unsaturated γ-lactones in plants are still unknown. Expoxidation, however, is believed to be the initial step in many biomodifications such as the transformation of squalene to steroidal compounds *via* squalene 2,3 oxide.[20] The large number of naturally occurring epoxide terpenoids also gives credence to this assumption. A reasonable pathway suggested by Geissman is illustrated in Scheme III.[21]

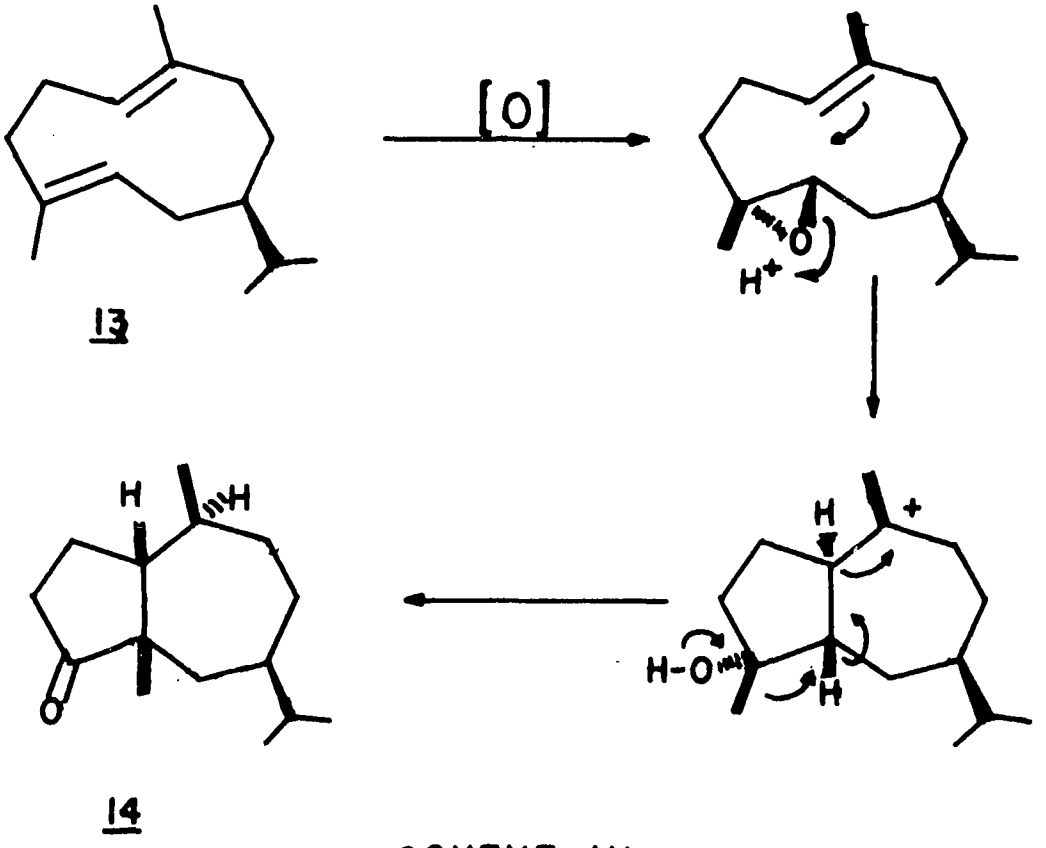


SCHEME III

In fact, trans-annular ring closure of the cyclodecadiene germacranolides to give the different structural types of bicyclic terpenoids is believed to proceed also *via* epoxide intermediates.[22]

The formation of guaianolides and pseudoguaianolides from 13 is realized by stereospecific cyclizations leading to the guaiane and pseudoguaiane skeletons. Pseudoguaianolides (14) may be formed

by a stereospecific rearrangement of the guaianolide skeleton (13) as shown in Scheme IV.



13

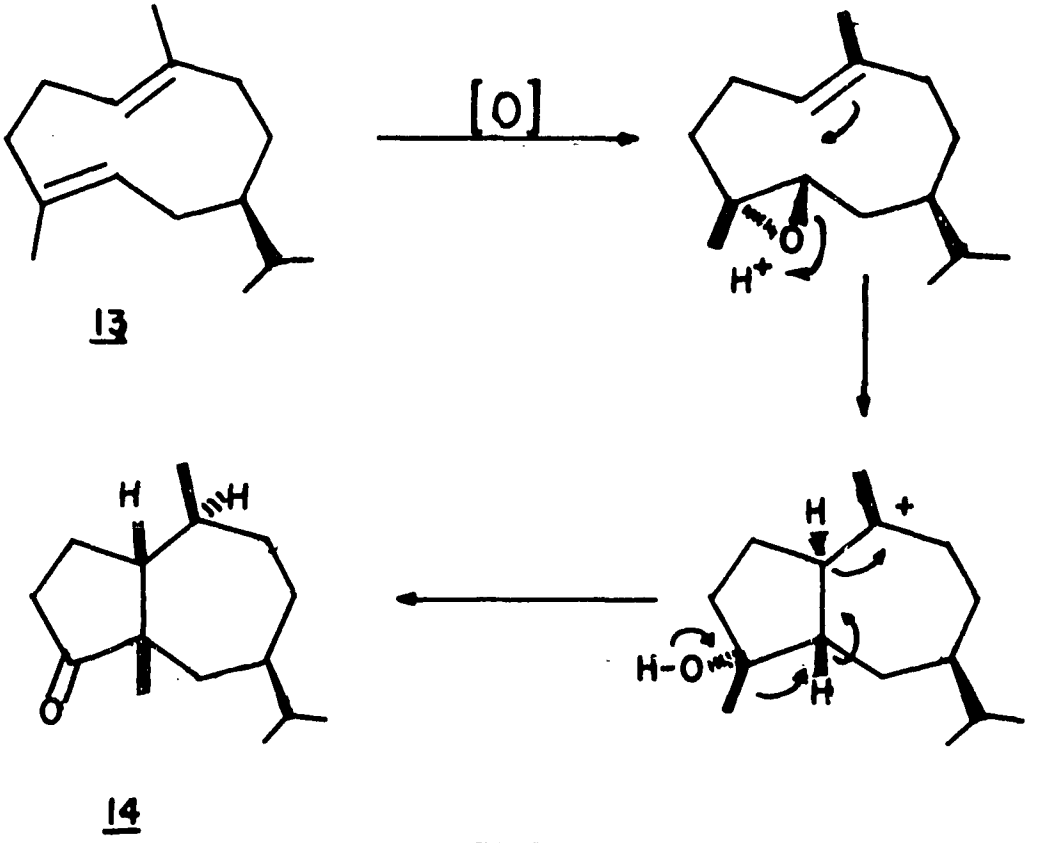
14

SCHEME IV

A summary of possible sesquiterpene lactone biosynthesis is given in Scheme V.

To date, only germacranolide type sesquiterpene lactones have been isolated from the genus Melampodium. For this reason a more detailed discussion of the germacranolide structure is important. It was realized by Kupchan, Kelsey and Sims (KKS)[23] that the germacranolide ring is symmetrical about the C_2-C_7 axis, and thus

by a stereospecific rearrangement of the guaianolide skeleton (13) as shown in Scheme IV.



13

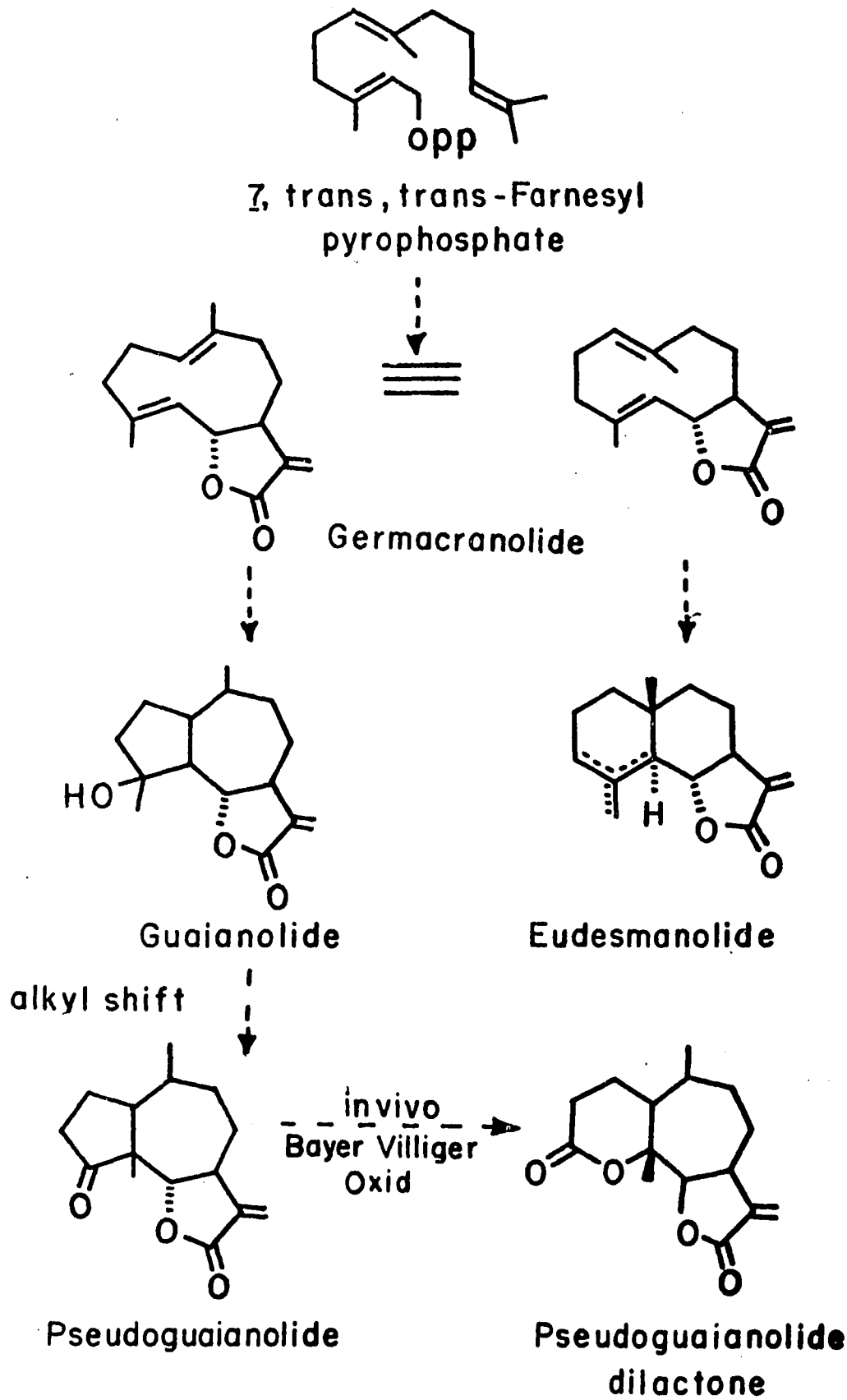
14

SCHEME IV

A summary of possible sesquiterpene lactone biosynthesis is given in Scheme V.

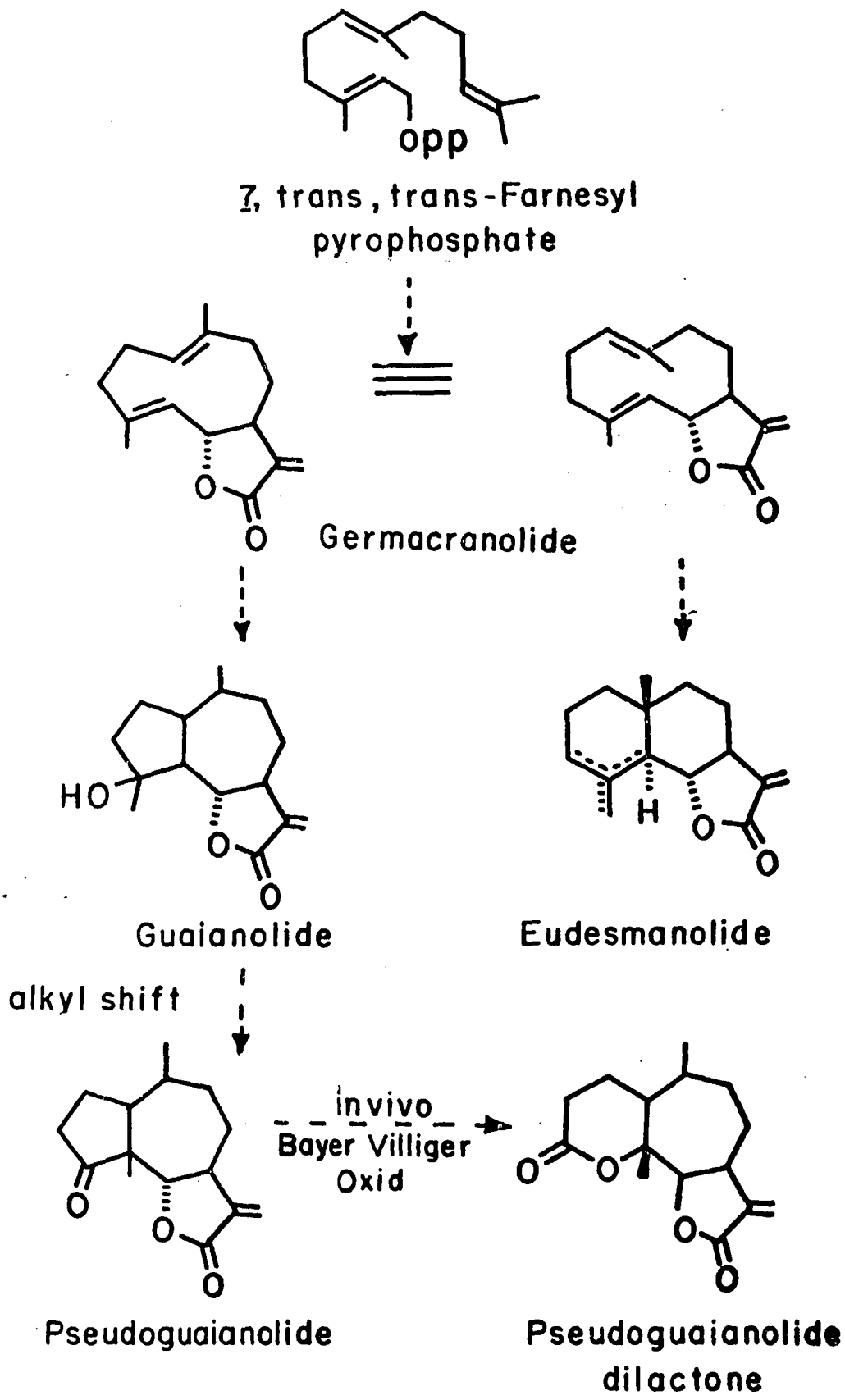
To date, only germacranolide type sesquiterpene lactones have been isolated from the genus Melampodium. For this reason a more detailed discussion of the germacranolide structure is important. It was realized by Kupchan, Kelsey and Sims (KKS)[23] that the germacranolide ring is symmetrical about the C_2-C_7 axis, and thus

Possible Biosynthesis of Sesquiterpene lactones



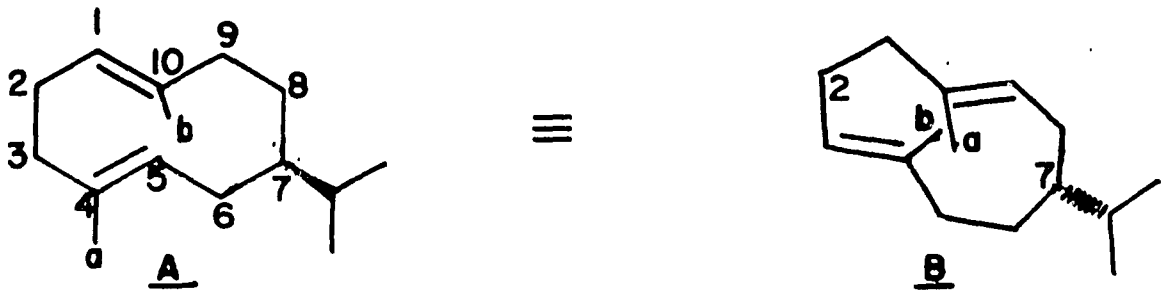
SCHEME V

Possible Biosynthesis of Sesquiterpene Lactones



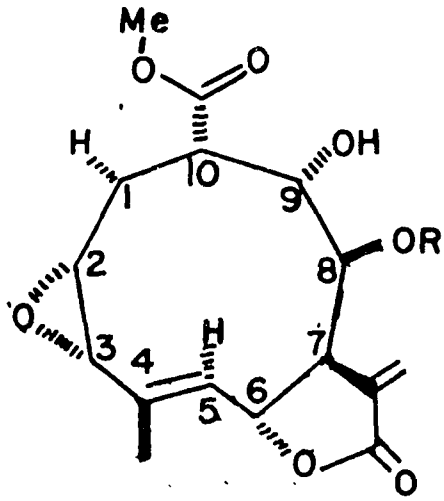
SCHEME V

it can be represented in two equivalent forms A and B. In A the

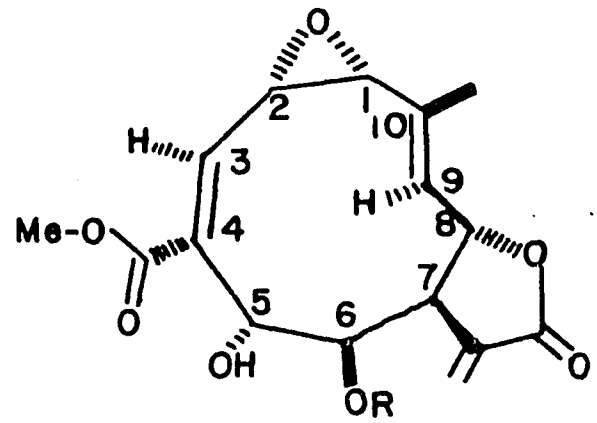


substituent at C-7 adopts a β-orientation whereas in B it is α-oriented. It has been proposed[24] that germacranolides be represented as in A in order to conform with Hendrickson's biogenetic generalization[14,15] that all sesquiterpenes have C-7 β-substituents (KKS rule). Indeed, sesquiterpenoids from plant sources generally follow this rule; it should be mentioned, however, that Weinheimer and coworkers[26] isolated from marine organisms a germacradiene with an α-oriented C-7 substituent. The latter findings demonstrate clearly that caution should govern the application of the C-7-β-rule and that the absolute configuration of a new natural product can only be determined by an absolute method (X-ray and/or neutron diffraction) or by chemical correlation with a compound of known absolute structure.

A typical example for the kind of problems involved in the structure elucidation of a germacranolide was demonstrated in the characterization of melampodin-A. From chemical evidence[27] two possible structural isomers, (15) and (16), were consistent with the KKS[23] conventions. It was only after the absolute configuration of melampodin-A was determined by single crystal X-ray diffraction (Rogers and Neidle)[28] and later by neutron diffraction (Watkins, Fischer, and Bernal),[29] that the structure of melampodin-A was shown to be 15.

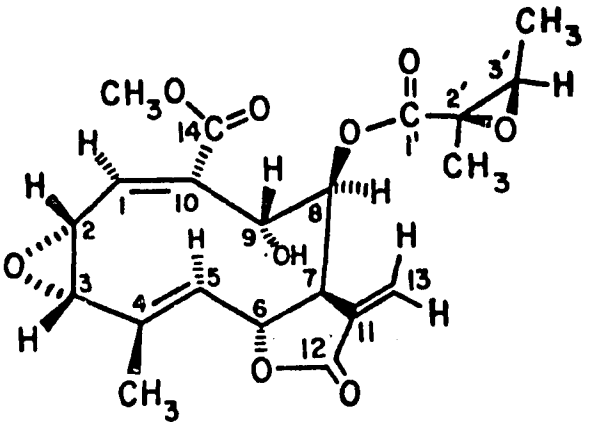


15



16

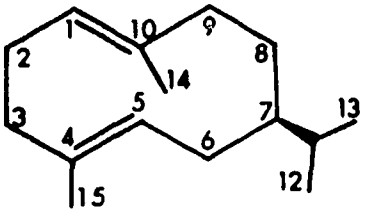
Rogers and Neidle then proposed a new convention[30] for the two-dimensional representation of three-dimensional germacranolide structures: Substituents at tetrahedral ring atoms use the conventional symbols (- - -, ———) to denote their α, β relationship to the plane of the ring and will be used exactly as in the sterols. The terms <u>cis</u> and <u>trans</u> when applied to endocyclic double bonds indicate the sequence of <u>ring</u> bonds. If the plane of such a double bond is approximately perpendicular to the macrocycle, one indicates which of its orientations occurs by using the above symbols to denote the orientation of its substituents α or β to the general plane of the macrocycle.[30] Thus, as in melampodin-A (<u>17</u>), the C-4(5) double bond is <u>trans</u> with a β-methyl substituent and an α-C-5 hydrogen while the carbomethoxy group at C-10 and the H-1 are α to the plane of the ring. Melampodin-A was the first example of a 1(10)-<u>cis</u>, 4(5)-<u>trans</u> germacranolide. This new structural feature



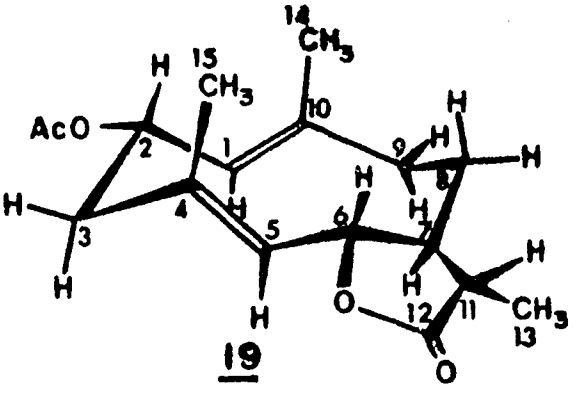
17

resulted in the reclassification of germacranolides into the following four subgroups:[31]

1. The germacrolides (18) possess trans double bonds at C-4(5) and C-1(10) positions and are the most common of the germacranolides. Those with a 7,6-lactonic ring closure exist in a stable conformation with the C-14 and C-15 methyl groups being syn-oriented toward the β-face of the medium ring; this is shown in the conformational representation for dihydrotamaulipin-A acetate (19).[32]

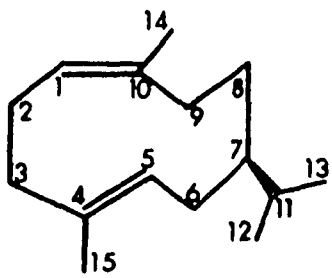


18

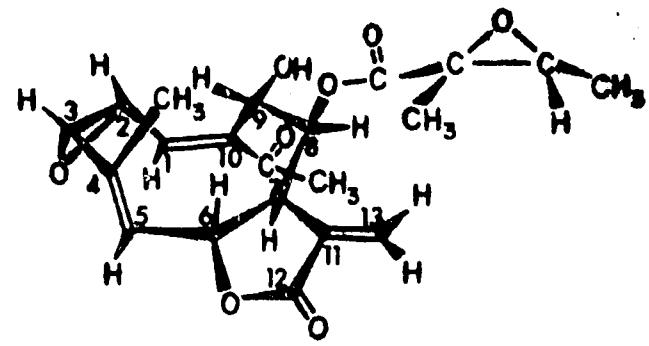


19

2. The melampolides (20) have a trans double bond at C-4(5) and at C-1(10) a cis configuration of the double bond. The anti-orientation of the carbomethoxy group at C-10 and the C-4 methyl group seems to be the typical conformation of this sub-group. A representative is the highly strained compound melampodin-A (21).[27-29]

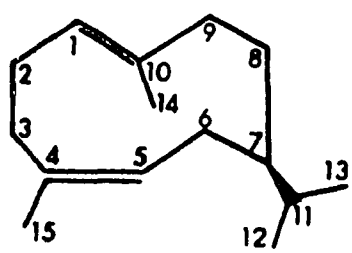


20

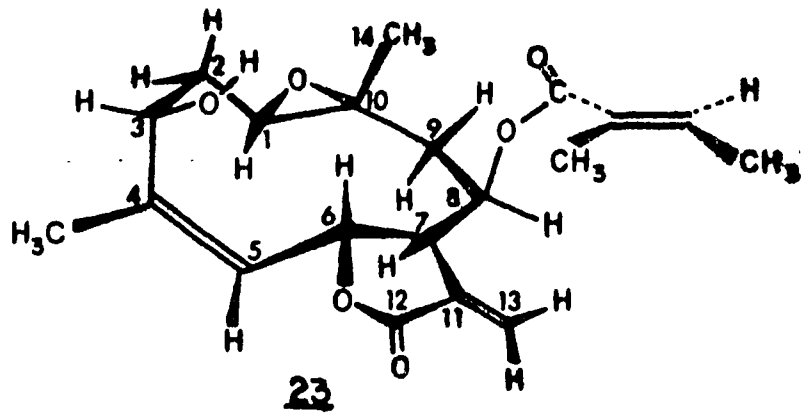


21

3. The heliangolides (22) have a cis,trans arrangement of their C-4(5) and C-1(10) double bonds, respectively. Their conformation is similar to that of the melampolides in that C-14 and C-15 are anti-oriented to one another but in opposite directions. Typical derivatives of the helianolides are heliagine (23),[33] eriflorin,[34] and Woodhousin.[35]

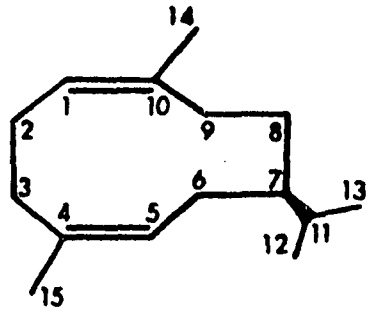


22



23

4. The fourth subgroup contains a cis,cis cyclodeca-1,5-diene ring system (24). Natural products belonging to this subgroup have not been found, although eupacunin[36] and liatrian[37] seem to be biogenetic derivatives of this subgroup.

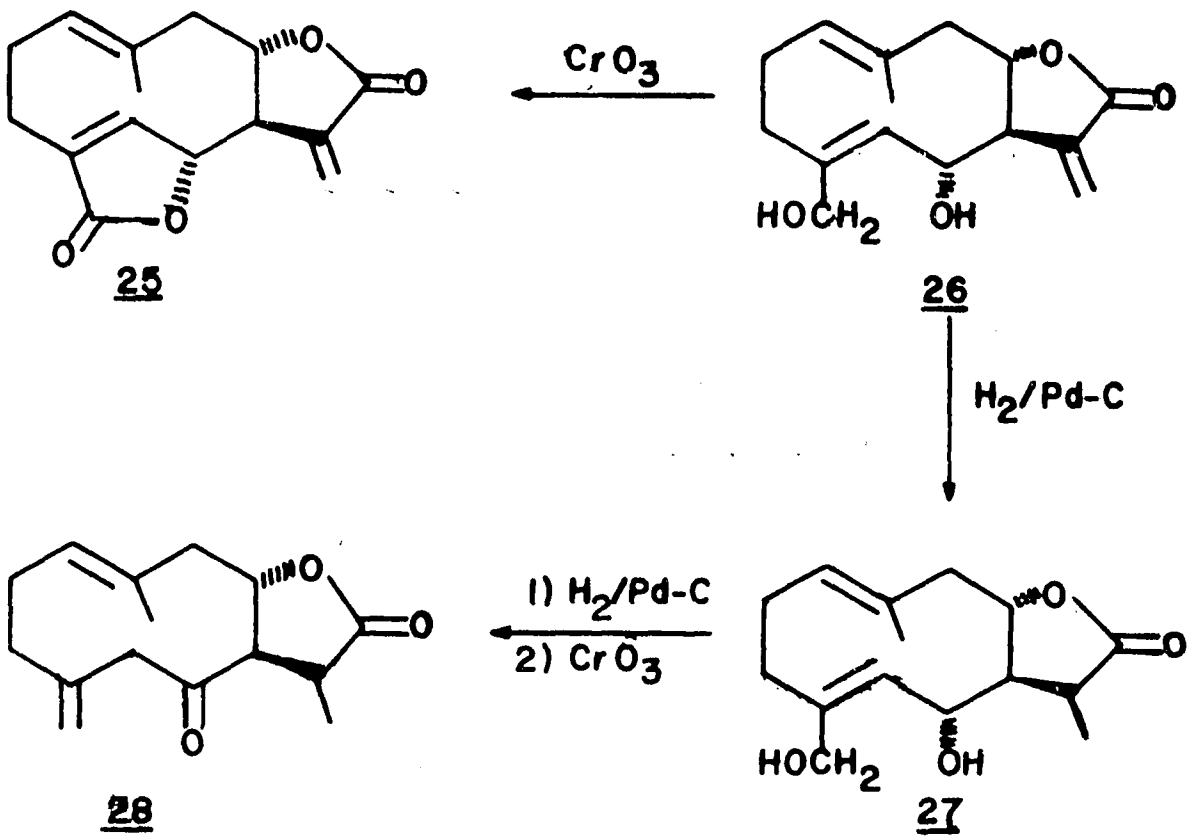


24

Germacranolides are usually lactonized at the C-6 or C-8 positions with additional oxygen functions found at C-2, C-3, C-5, C-6, C-8, or C-9. Either or both of the methyl groups may be oxidized to the hydroxyl, aldehyde or carboxyl functions while either double bond may be reduced or transformed into epoxide groups. Germacranolides are believed to be the biogenetic precursors of the other classes of sesquiterpene lactones (Scheme V) for some germacranolides are readily converted in vitro into other skeletal types either by acid catalysis, thermolysis or photolysis. As with other sesquiterpene lactones, most of the chemical transformations that germacranolides are known to undergo were first described in the course of structure determination studies. Selected examples of typical reactions involved in the structure elucidation, conformational analysis, and chemistry of germacranolides are briefly presented.

Hydrogenation and Oxidation

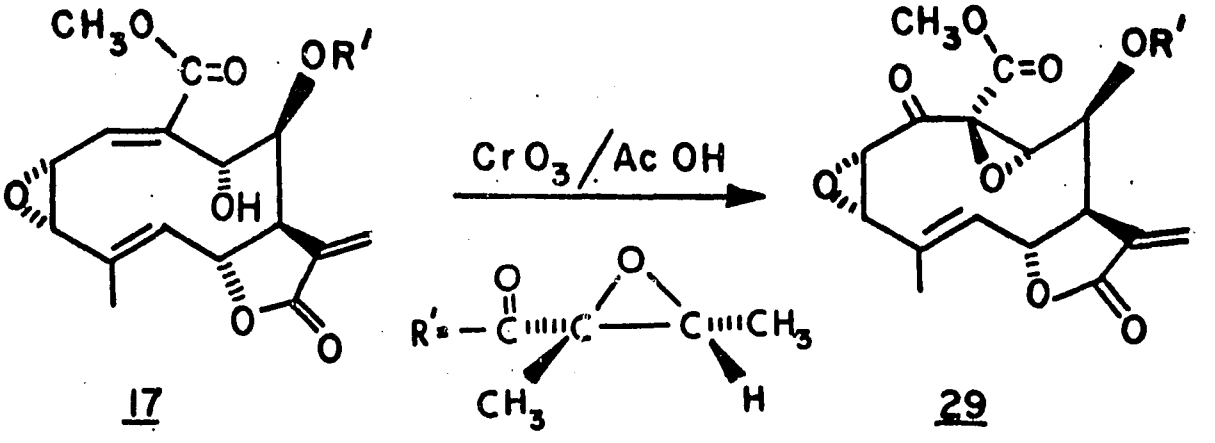
These reaction types are primarily employed to make derivatives of the unknown natural products in order to change their NMR parameters and mass spectral fragmentation patterns. Hydrogenation (the ease or lack of it) may be used as chemical evidence for the conformation and/or stereochemistry around the double bonds while oxidation can give additional information concerning the nature of hydroxyl substitutions. The following are examples of such reactions:



25 26 28 27

SCHEME VI

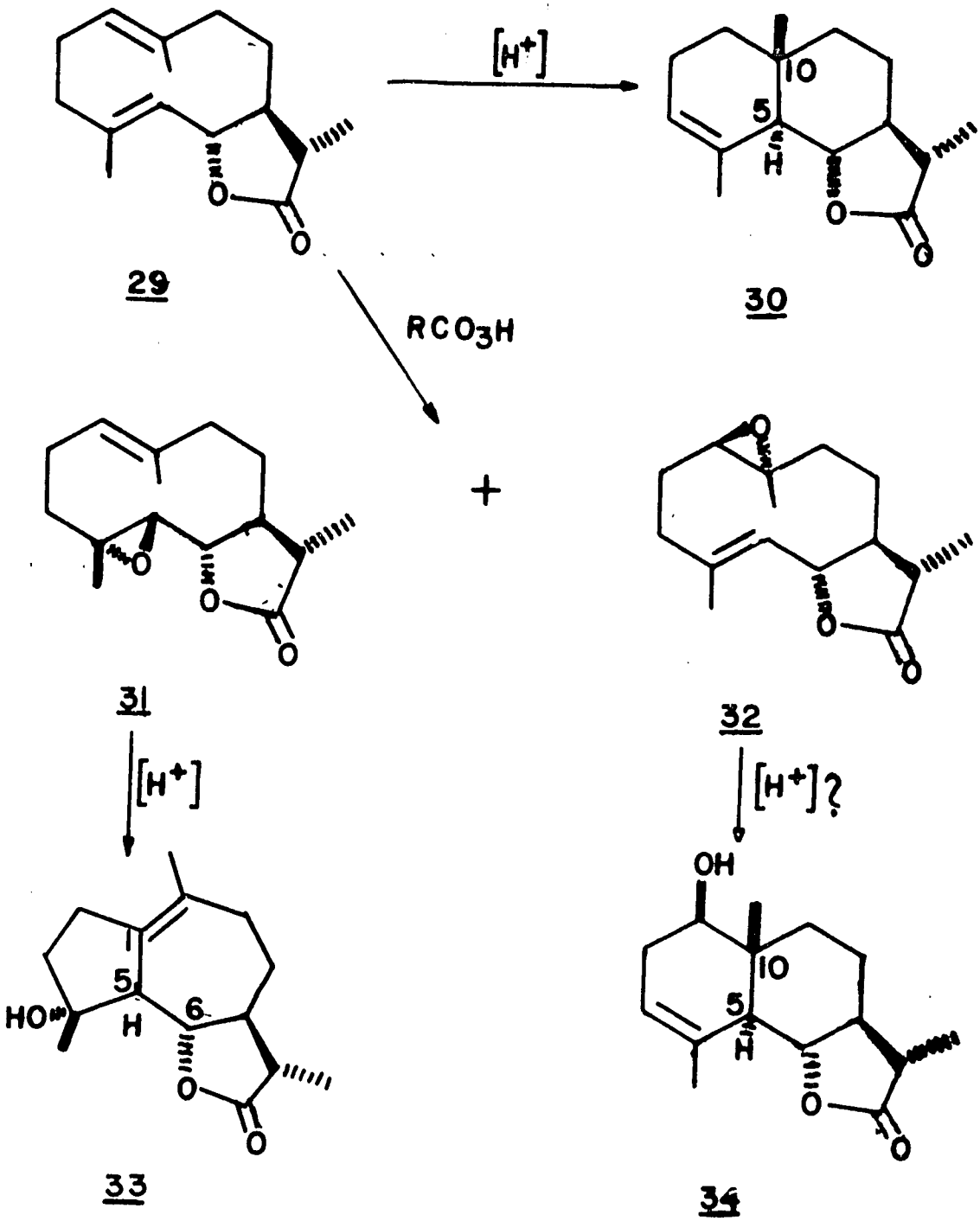
Artemisiifolin (26)[38] is oxidatively transformed into isabelin (25)[39] by using CrO_3 and is reductively transformed into salonitolide (27)[40] using hydrogen over a Pd-C catalyst. Hydrogenolysis of 27 followed by CrO_3 oxidation gives the keto-lactone 28 (Scheme VI). The oxidation of melampodin-A (17) with chromium trioxide in glacial acetic



acid or with Jones' reagent produced the keto compound 29.[41] Ring strain and steric factors in the medium ring seem to be the major reasons for a change in the mode of the oxidation reaction.

Acid-catalyzed Cyclizations

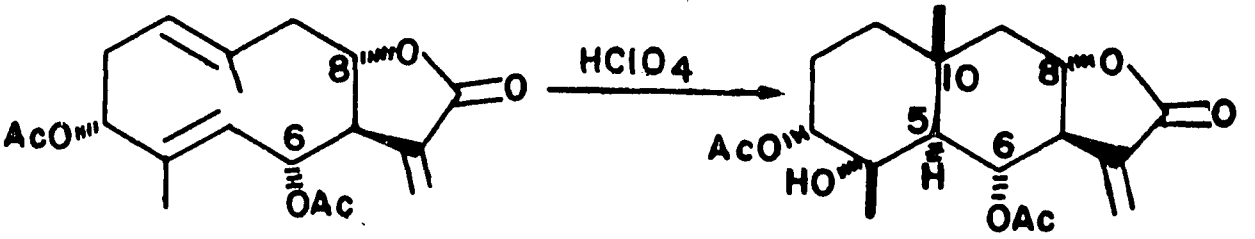
Another useful approach to the stereochemical characterization of germacranolides is afforded by the acid-catalyzed cyclizations of these molecules. The conversion of dihydrocostunolide (29) to the eudesmanolide, dihydro-α-cyclocostunolide (30)[42] is a good example (Scheme VII). Epoxidation of 29 gave a mixture of dihydroparthenolide (31) and its C-1(10) epoxy isomer (32). A further acid-catalyzed cyclization of 31 yielded the guaianolide 33[43] while one



SCHEME VII

would predict that the reaction of 32 would give the eudesmanolide 34. These reactions point out how the position of the epoxide group can direct the acid-catalyzed cyclizations of germacranolides.

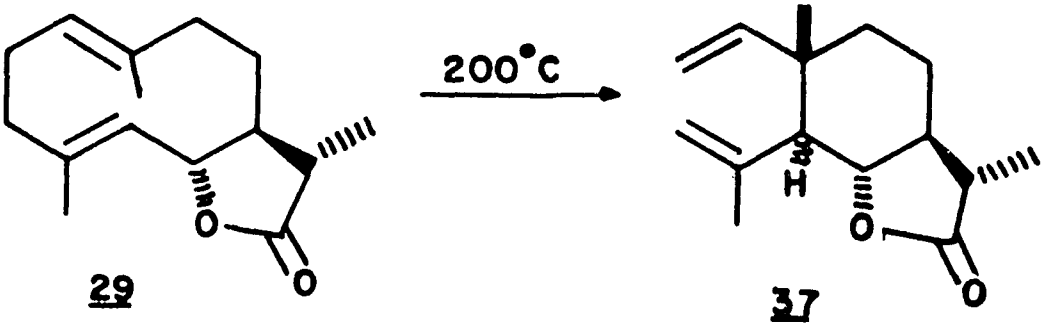
Chamissonin diacetate (35) has been cyclized to cyclochamissonin diacetate (36) using perchloric acid. This transformation gave important information in connection with the structure revision of chamissonin.[44] The resulting eudesmanolide contains a trans-decaline ring with a C-10 methyl group. To date, all eudesmanolides derived from germacranolides by acid treatment have a trans-decaline ring.[45,46]



35 36

Cope Rearrangement

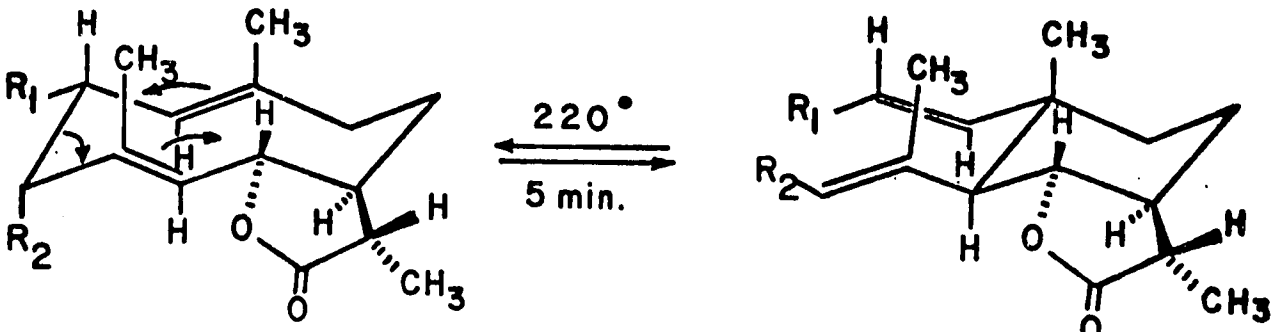
Germacranolides which are 1,5-dienes undergo a Cope rearrangement at elevated temperatures (150-250°) to give equilibrium mixtures of the starting material and Cope products. Saussurea lactone (37), in fact, was initially thought to be a natural product until it



29 37

was shown to be an artifact of dihydrocostunolide (29) which had formed during the steam distillation of the plant extract.[47]

The in vitro Cope reactions of germacranolide lactones require saturation of the α,β-unsaturated double bond in the γ-lactone since the exocyclic methylene function is unstable at higher temperatures. When the dihydroacetate derivative of tamaulipin-A or B (38 or 39, respectively) was pyrolyzed an equilibrium mixture of the starting material and the Cope product 40 or 41, respectively were obtained.



38, R_1 = OAc; R_2 = H
39 R_1 = H; R_2 = OAc

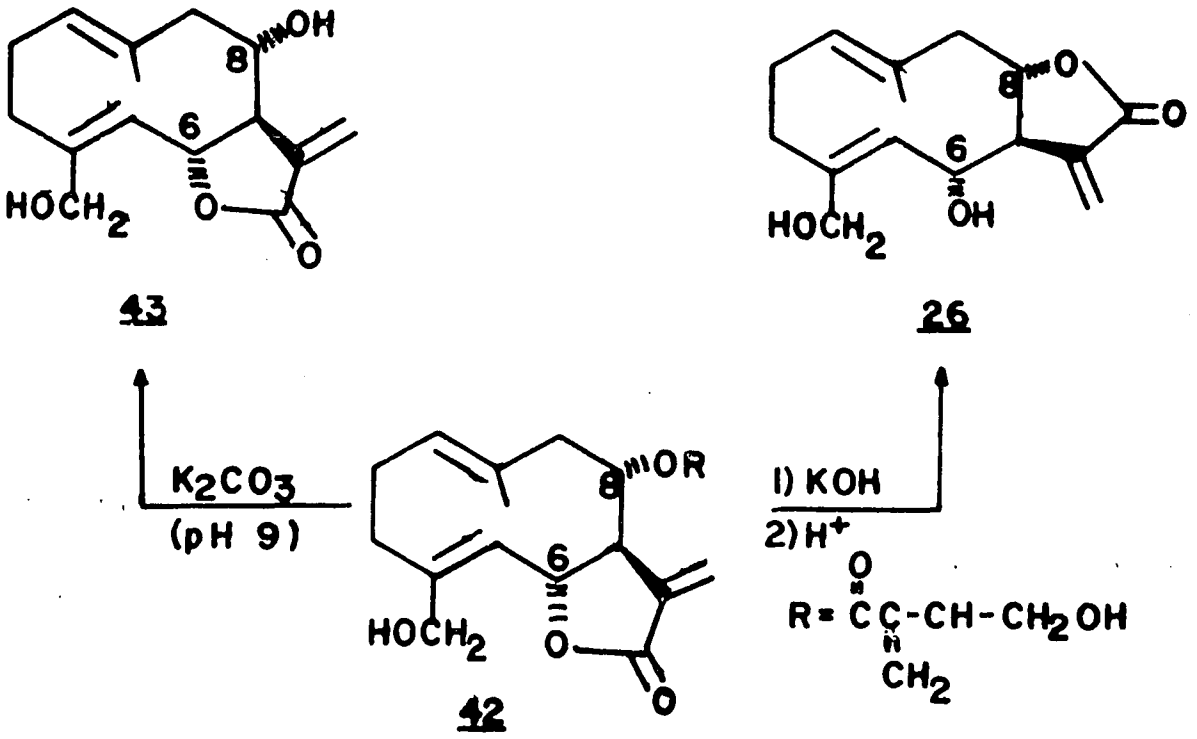
40, R_1 = OAc; R_2 = H
41 R_1 = H; R_2 = OAc

The Cope rearrangement of 1,5-dienes has been shown to proceed through a quasi-chair transition state[48,49]. The stereochemistry of the two newly formed asymmetric centers (C-5 and C-10 in 40) indicate that the conversion of dihydrotamaulipin-A acetate to 2-acetoxysaussurea lactone (40) must also involve a chair-like transition state. Consequently, the conformation of dihydrotamaulipin-A acetate can be illustrated as 38. Additional experimental evidence concerning the conformation of 38 in solution at room temperatures and below was provided by nuclear Overhauser effect studies.[32] Irradiation of the C-4 methyl signal increased the

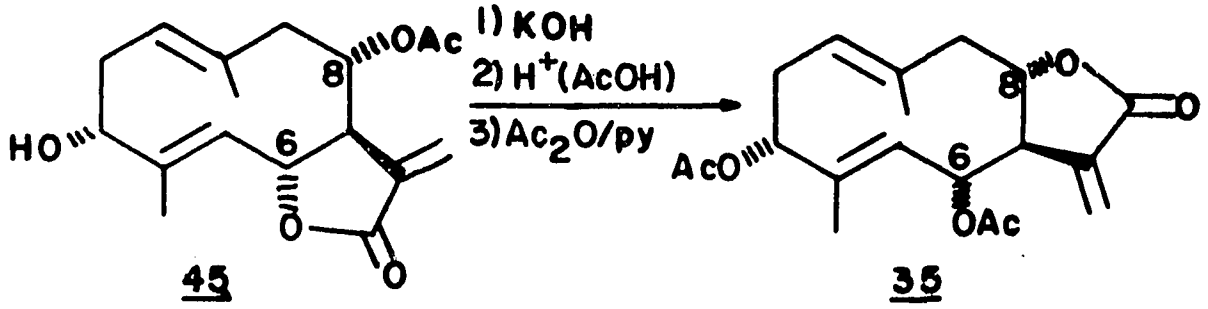
intensity of the H-2 and H-6 proton signals suggesting a syn-orientation of these groups. Irradiation of the C-10 methyl group also produced a positive response on the H-2 signal again indicating a syn-arrangement. These results indicate that the C-4 and C-10 methyl groups have the same direction in space and thus have a syn-orientation. Therefore, the planes of the olefinic linkages should be almost perpendicular to the plane of the carbocyclic ring and the double bonds should have a crossed orientation as shown in 38.

Hydrolysis and Relactonization

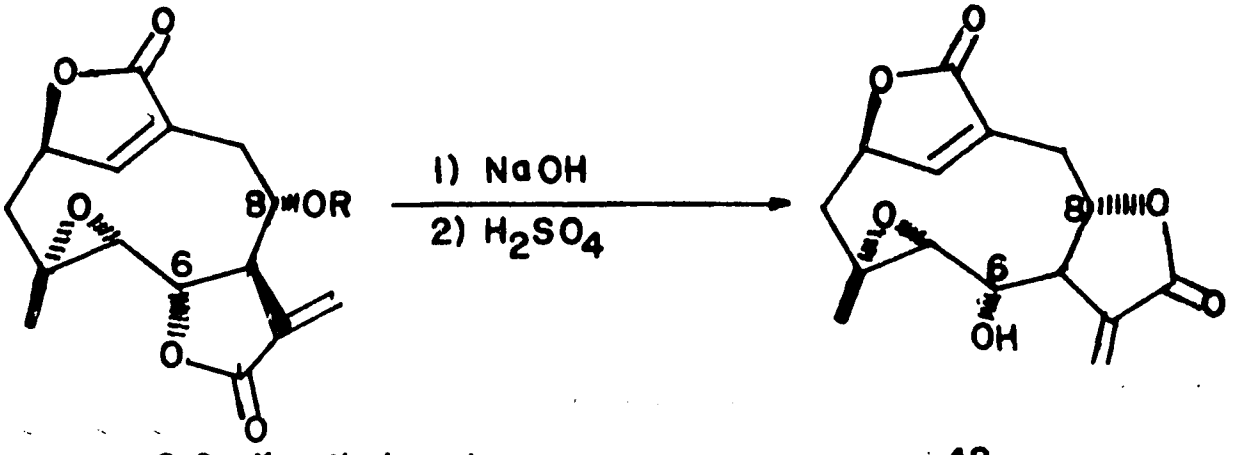
Most germacranolides contain C-6 and C-8 lactonizable oxygen functions. This feature contributed to the incorrect structural assignment for salonitenolide (43), since it was generally accepted that germacranolides with C-6 and C-8 oxygen functions could, after alkaline hydrolysis, relactonize to either the C-6 or C-8 positions. Recently, however, a general relactonization rule for germacranolides that contain both C-6 and C-8 lactonizable oxygen functions was described: "Upon strong alkaline treatment followed by acidification, this type of germacranolide (with an α-hydroxy group at C-8) relactonizes to C-8."[50]



Thus, hydrolysis of cnicin (42) and chihuahuin (45) under conditions that open the lactone ring gave, upon acid treatment, a C-8 lactone. Cnicin can also be hydrolyzed under conditions that do not open the lactone function but hydrolyze the attached ester group at C-8; this method gives salonitenolide (43).[50]



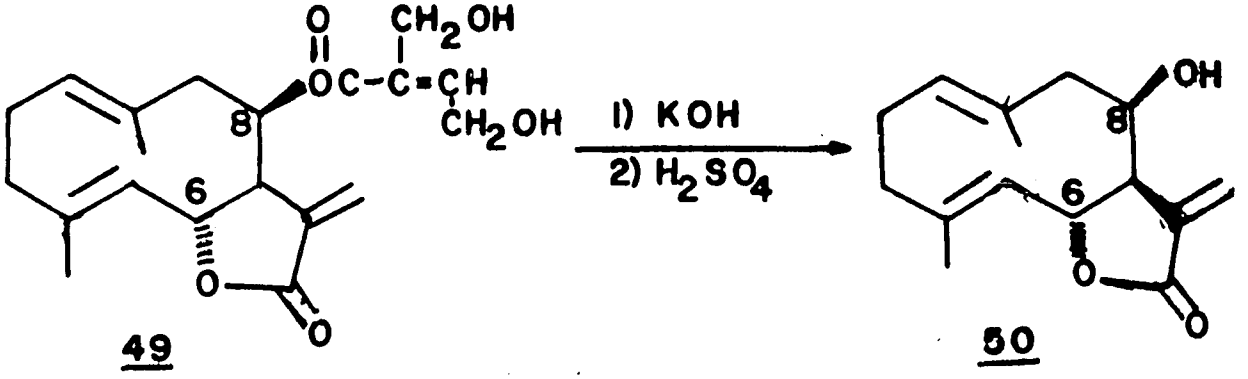
Furthermore, it has been shown in the germacranolide dilactones elephantin (46) and elaphantopin (47) that a C-8 lactone elephantol (48) is formed after alkaline hydrolysis and relactonization under acidic conditions.[51] In contrast, eupatoriopicrin (49) which contains a β-oriented oxygen function at C-8 has been shown to reform the C-6 lactone eupatolide (50) after relactonization.[52]



46 R = β,β-dimethylacryl

47 R = methylacryl

48

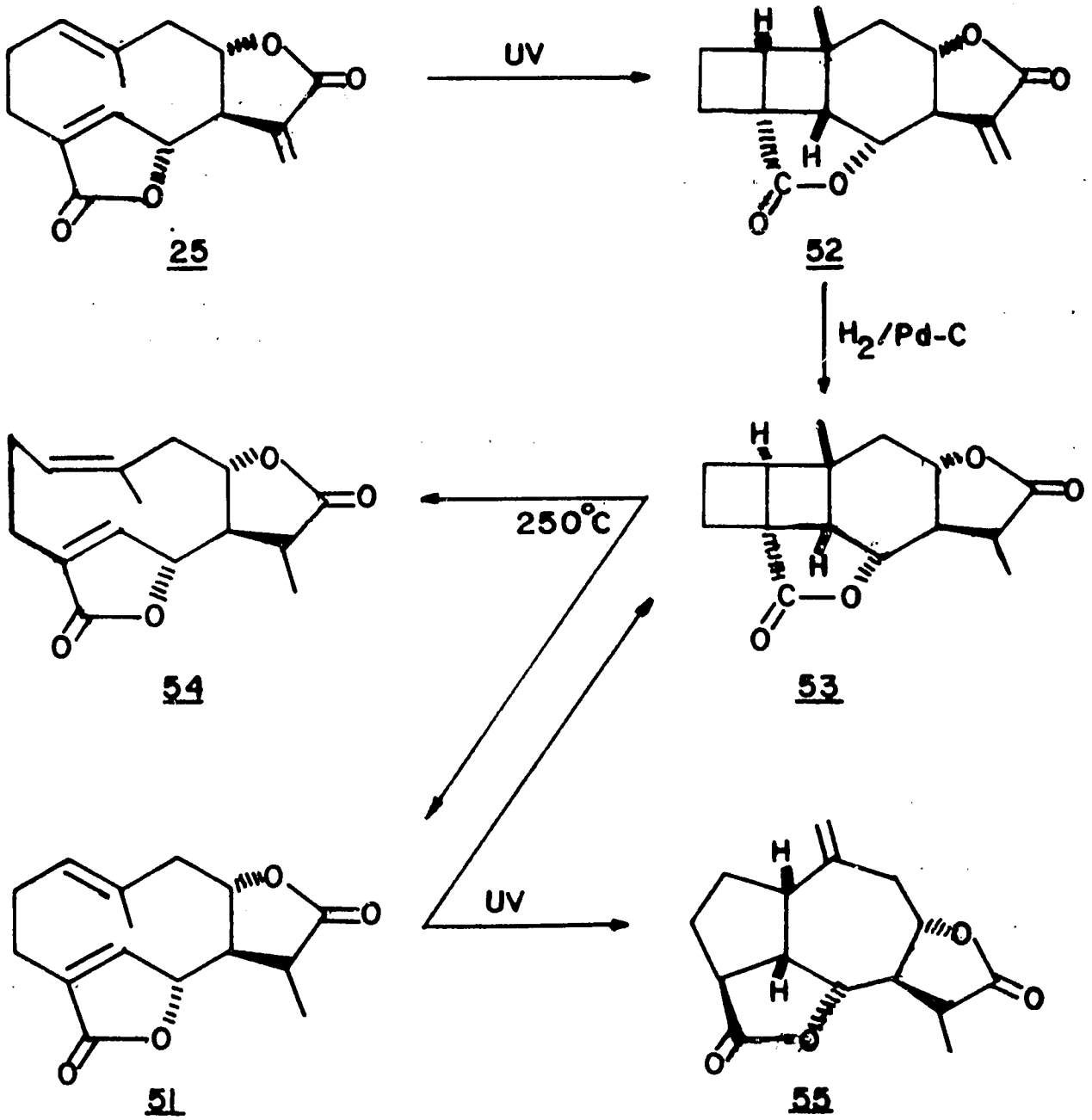


49

50

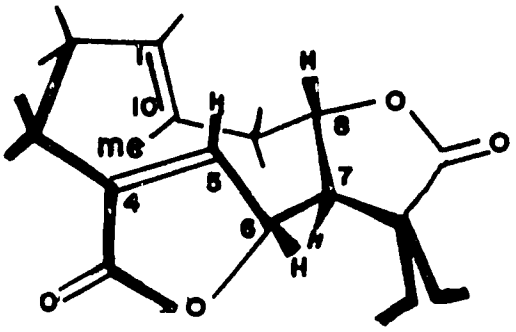
Photochemical Transformation

The best examples of the photolysis of germacranolides are those concerning isabelin (25) and dihydroisabelin (51):

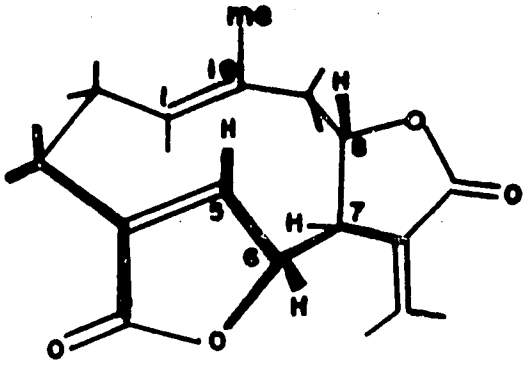


SCHEME VIII

Because one of the double bonds in the cyclodecadiene ring is activated by the carbonyl function, it was predicted that the photocycloaddition of isabelin (25) was possible.[53] These transformations were considered of particular interest because they would help establish the notion that the nature of the photoproducts for such systems are controlled in part by the conformation of the cyclodecadiene ring. At room temperature, isabelin exists in solution in a 10:7 ratio of two NMR-distinguishable conformers X and Y.[39]



X



Y

However, the ratio is altered to 1:5 when isabelin is dissolved in $CDCl_3$ precooled to -50° and the NMR spectrum recorded at that temperature. Conformer Y, which is the minor conformer in solution at room temperature, appears to be the only conformer present in the solid phase. In conformer X, the endocyclic double bonds are in a crossed orientation, whereas in Y, these double bonds are parallel. Both conformers have general geometrical features required for a [2π + 2π] cycloaddition; however, molecular models indicate that such a reaction is structurally inhibited for conformer X.

Isabelin (25) after irradiation in benzene at 253.7nm was converted to photoisabelin (52). This clearly resulted from a

[2π + 2π] cycloaddition process involving conformer Y. Dihydroisabelin (51), in contrast, when photolyzed under the same conditions gave the ene-photo product lumidihydroisabelin (53) (minor). The photolytic formation of lumidihydroisabelin is apparently due to the fact that dihydroisabelin (51), unlike isabelin, exists in solution as a single conformer in which the double bonds have a crossed orientation (conformer X).[53]

The previously described reaction types are commonly used in establishing the structures and conformations of sesquiterpene lactones from various plant collection of a particular plant species. In the past, structure elucidation was the primary objective of the researcher; now, however, this procedure encompasses only part of the objective with biochemical systematics becoming just as important. Since the biosynthesis of secondary plant metabolites is genetically controlled, the correlation of the number and types of natural products in general and sesquiterpene lactones in particular found in each collection may provide some kind of judgement as to the distinction of species within a particular genus. Evolutionary processes may also be deciphered by these studies. In short, this is the rationale behind biochemical systematics.

Chemotaxonomy (or biochemical systematics) is now well established as an interdisciplinary field of study spanning simultaneously all of phytochemistry and plant systematics.[54-57] As in other areas of scientific research, the degree of acceptance or rejection of the methods employed in biochemical systematics will depend upon the reliability of the chemical data. In 1967, Alston

made the following assertions as to the matter of infraspecific variation which is an important aspect of the problem of reliability:

> "In modern systematics, variation is often a valuable asset. Although some species are more variable than others, and in some instances there is extreme polymorphism, variation in general is accepted as a biological fact that may under certain conditions be essential to allow the effective study of populations, the study of incipient speciation, or of ecological factors and other important problems. The study of genetics is absolutely dependent upon phenotypic variation. For these and other reasons, it should not be either surprising or discouraging to find chemical variation existing even within a species. Since an important element of any scientific work is predictability, the question of variability must be evaluated according to this criterion. If chemical variation in plants is so excessive, so capricious, and so generally immune to analytical interpretation that predictability is essentially lacking, then such data hardly can be utilized scientifically at all, certainly not by systematists. But, if variation in secondary products is generally responsive to various factors similar to those factors which govern the morphology of a species, then chemical variation can be described in such a way as to introduce limits to the variation and to discern its pattern and thus, of course, to allow predictability. Since the latter alternative is nearly axiomatic, in my opinion, then the problem is merely to become familiar with chemical variation, its origin and factors affecting it, and its limits and its meaning, if possible, just as any good systematist would attempt to study morphological or cytological variation."[54]

In the above passage, two ideas are particularly important to note: a) "infraspecific variation" is primarily concerned with secondary plant products, and b) the understanding of chemical variation is tantamount to the understanding of infraspecific variation. Continuing on these ideas, it is known that all plant species produce

some unique secondary compounds, often in large quantities.[58] Many of these compounds are known to serve as defensive agents against herbivores and other predators. Sesquiterpene lactones may serve in this capacity as a result of their bitter and toxic principles. Other secondary plant compounds regulate biochemical processes and mediate a variety of developmental responses, while some are metabolized and are therefore found only in steady state concentrations.[59]

The phenomenon of chemical variation is studied by deciphering the chemistry of geographical races (that is, the chemistry of different populations of a given or closely related taxa) because it is well established that most if not all species have been able to radiate geographically and ecologically through dispersal and adaptations which usually exhibit some sort of ecotypic variation.[59] Some of this ecotypic chemical variation represent only minor qualitative differences while in other cases this variation appears to represent dramatic structural change as a result of mutational loss: that is, the ability to synthesize certain compounds has been lost. It has been recognized that chemical agents are of major significance in the adaptation of species and organization of populations.[58,60] Therefore, the interactions of plants, animals, and microorganisms with each other and with ecological and environmental elements of a given ecosystem will determine much of the secondary chemistry of a plant population. Indeed, as pointed out before, some investigators believe that most of the natural product chemistry of a particular plant taxon serves as a defensive system against predators. It still must be recognized, however, that despite even drastic changes

in the ecosystem a particular structural theme (regardless of the class of compounds) will almost always continue to persist in all populations of a taxon or populations of closely related taxa. In general, the sort of chemical ecotypic variation that is encountered on going from population to population involves the number of compounds, degree of oxygenation, methylation and glycosylation, and such other structural modifications as rearrangements and cyclizations.[59] These modifications may result from the fact that the new ecosystem requires a physiologically different organism and this biochemically different plant then produces, secondarily, these minor modifications.

Genetic drift, occurring in geographically isolated populations, is also a potential source of genetic variation which could lead to chemical races. Other factors which have contributed to the origin of the chemical races present in today's taxa are major geological and climatic events such as the shifting of land masses, formation of mountain chains, and periods of glaciation. It should be mentioned, however, that most populational chemical variation will be understood only when the detailed physiological basis for the differentiation is explained.

There are many examples reported in the literature in which the infraspecific variation of sesquiterpene lactones is a well established phenomenon.[54,61-64] Perhaps one of the most informative studies was the detailed comparative chemical, morphological, and in some instances cytological analysis of the essentially New World genus Ambrosia.[62] Most Ambrosia species[62-64] are rich in sesquiterpene

lactones; in fact, the genus is known to have more than 30 different sesquiterpene lactones distributed among the five different skeletal types. The Ambrosia species are characterized as shrubs or perennial or annual herbs and are commonly referred to as ragweeds, plants which are perhaps best known in North America for their contribution of allergenic pollens to the atmosphere. There are now 40 recognized species of Ambrosia and Payne has suggested a scheme illustrating the evolutionary relationships of the main North American species.[62,65]

A representative study of a particular species of Ambrosia is found in a study initiated by N. H. Fischer and completed by W. Renold with regard to the heterogenous species, A. confertiflora, a perennial which is wide-spread in the southwestern part of the United States and northern Mexico.[66] The sesquiterpene lactone data for more than 252 populations of this species serve to illustrate both the chemical complexity which a given species may exhibit and the way populational data can be used to detect evolution at work. Renold has reported that the distributional data for the sesquiterpene lactones (Figure 1) indicate that the species contain at least four mutually exclusive races:[66]

1. The tamaulipin chemical race (★) is the most widespread and ranges from the state of Tamaulipas, Mexico across northern Mexico to the state of Sinaloa, Mexico on the Pacific coast.
2. The confertiflorin chemical race (●) characterizes the species throughout central, south, and west Texas.
3. The psilostachyin-type chemical race (✱) is concentrated

in west Texas with some populations scattered throughout the state of Coahuha, Mexico.

4. The chihuahuin chemical race (■) is mainly restricted to the state of Chihuahua and the northern part of Durango, Mexico.

Even though these races were found to occur for the most part in distinct geographical regions; the situation was too complex to allow a generalization.

Chemical Races of *Ambrosia confertiflora* DC.

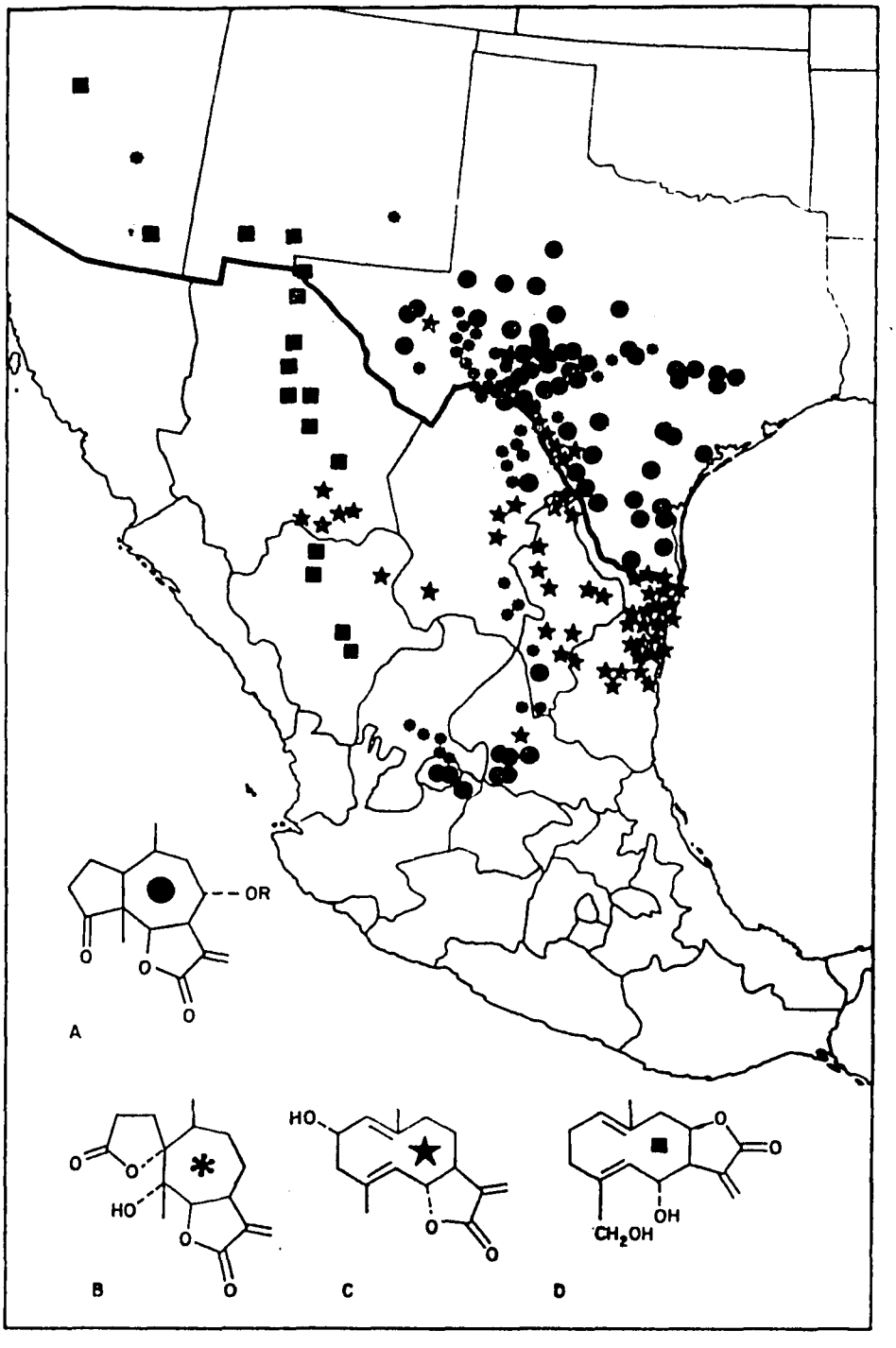


FIGURE 1

Part I of this dissertation presents the chemistry involved with the isolation, structure elucidation, chemical modification, and conformational considerations of the sesquiterpene lactones found in the genus Melampodium (Compositae).

Part II concerns the biochemical systematic aspects of the various plant populations which were collected throughout the southwestern part of Texas and northern Mexico.

Part III contains the experimental reactions, methods, and data.

PART I

The Isolation, Structure Elucidation, and Chemistry of Sesquiterpene Lactones from *Melampodium* (Compositae)

The genus Melampodium belongs to the family Compositae (the sunflower family) and is a tropical and subtropical genus generally distributed over Mexico and Central America with four species located in the southwestern United States and three species scattered in Colombia and Brazil. Recently the genus Melampodium was revised by Stuessy to include 37 species of which 34 have yellow-rayed flowers and the other three having white flower petals.[67,68] This dissertation deals primarily with the white-rayed complex, which are tap-rooted perennials found in the drier regions of northern Mexico and adjacent southwestern United States.

In this section, the structure elucidation and chemistry of a number of sesquiterpene lactones isolated from selected populations of M. cinereum, M. argophyllum, and M. americanum are described. Initially this investigation was directed toward the populational analysis of M. cinereum alone, but when the rare species M. argophyllum was found in the mountains of northern Mexico it was decided that this species should also be incorporated into our studies. The structure elucidation of a sesquiterpene lactone isolated from M. americanum is also included, for in contrast to earlier reports,* this is the first sesquiterpene lactone isolated from a yellow-rayed species of Melampodium.

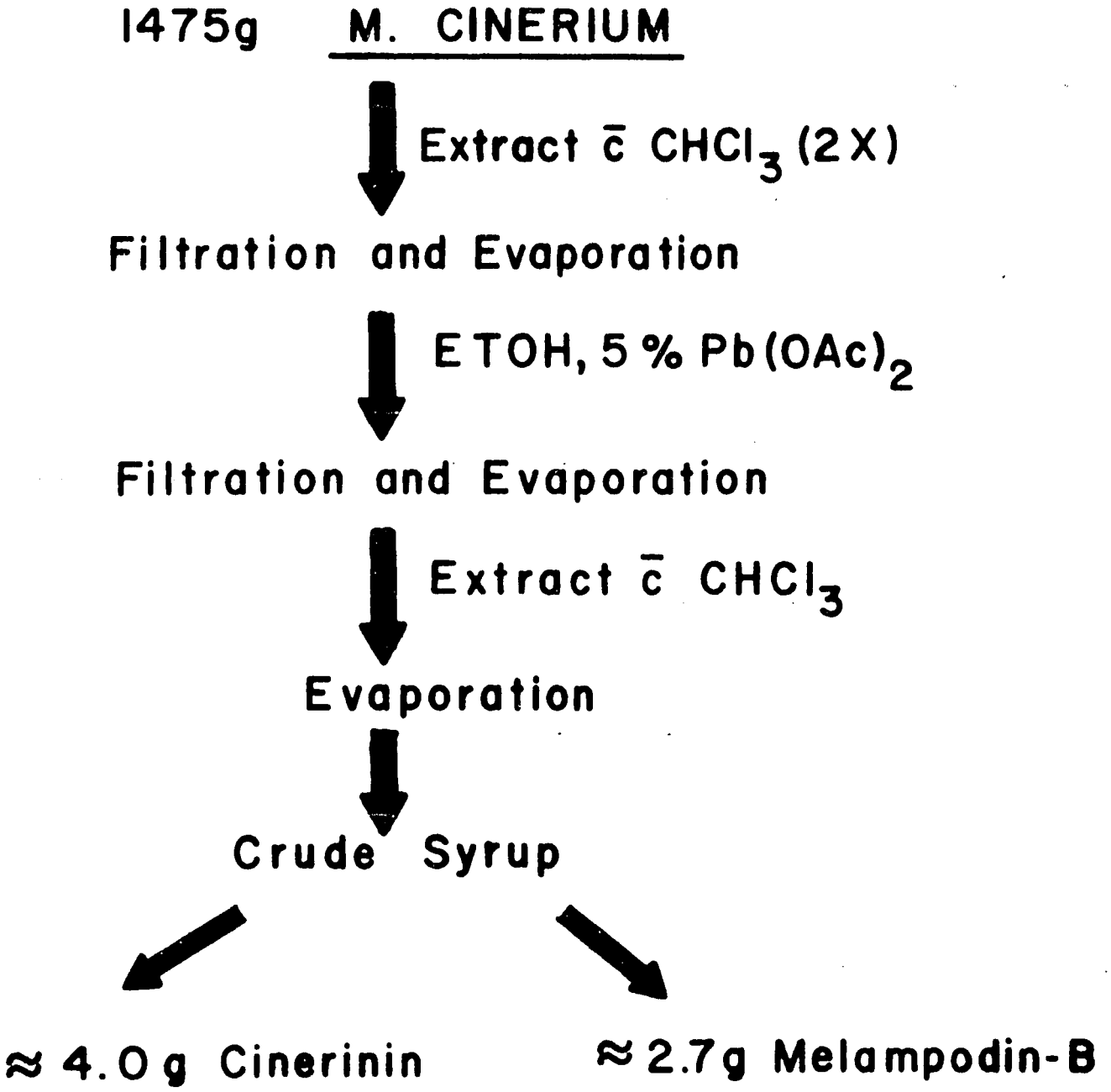
*A number of extracts from different yellow-rayed species of Melampodium contained no sesquiterpene lactones. (N. H. Fischer, unpublished data)

Isolation of Sesquiterpene Lactones

A number of different procedures have been used for the isolation of sesquiterpene lactones. The method of choice is usually determined by the type of natural products present in a particular collection. In this investigation, the following procedure was used for all populational collections (Scheme IX).

1. The weighed dried leaves were ground in a Waring blender and extracted with chloroform. After two hours the slurry was filtered and the leaves were re-extracted with chloroform. Chloroform was chosen for the extractions since terpenoid type natural products show reasonable solubility and it has a low boiling point for rapid evaporation.
2. The combined chloroform extracts were evaporated and the residual crude syrup was dissolved in ethanol and treated with a 5% aqueous lead(II) acetate solution (1 ml. of ethanol and 1 ml. of lead(II)acetate solution per 1 g. of dried leaves). The lead(II)acetate solution precipitates most phenolic compounds such as flavonoids, as well as chlorophylls.
3. The ethanol/water mixture was allowed to stand for about one hour and then filtered through filter aid (Celite). The ethanol/water filtrate was evaporated to approximately one-half of its original volume,

ISOLATION of SESQUITERPENE LACTONES



SCHEME IX

and then extracted three times with appropriate amounts of chloroform.

4. The combined chloroform extracts were dried over magnesium sulfate, filtered, and evaporated leaving the crude terpenoid-containing extract.

At this point, the various plant extracts were analyzed by thin layer chromatography and pmr as to their possible sesquiterpene lactone content. In some instances, sesquiterpene lactones crystallized from the crude extract after standing for approximately two weeks. In other cases, column chromatography was used to isolate the sesquiterpene lactones. The exact conditions which were used in the isolation of sesquiterpene lactones will be described in their respective experimental sections (PART III).

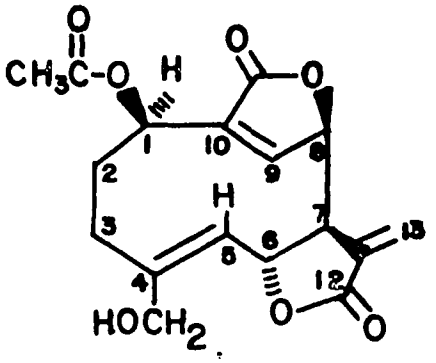
MELAMPODIN-B

An extensive analysis of over 80 populations of M. leucanthum (a white-rayed species) for their sesquiterpene lactone content was carried out by Mr. Russell Wiley and Mrs. Carolyn Fishback in our laboratory. This investigation has led to the isolation and structure elucidation of a number of new and novel sesquiterpene lactones in which melampodin-A (17) was chosen as a bench mark compound for this type of germacranolide, the melampolides.[27,29,31]

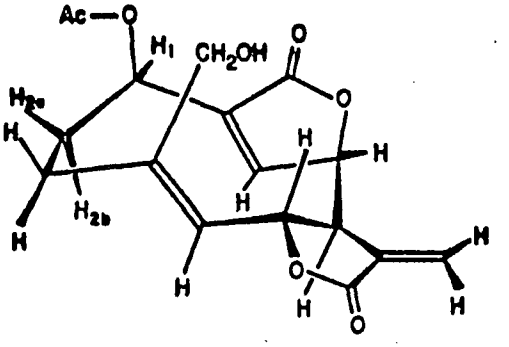
Another structural type of germacranolide, the dilactone melampodin-B (56), was isolated by Wiley from a single population of M. leucanthum found in west Texas.[69] Due to the limited amount of material the structure of melampodin-B was mainly deduced on the basis of spectral data using 300MHz ^{1}H nmr ^{13}Cmr analyses. With the exception of the preparation of melampodin-B acetate, no further chemical studies were carried out on melampodin-B.

It was recognized very early in my study of the chemical constituents of M. cinereum that melampodin-B (56) represented the major component in this species and that a number of new structurally related sesquiterpene dilactones also occurred with 56. For this reason melampodin-B was chosen as a model compound in the structure elucidation of other new sesquiterpene lactones from M. cinereum. Since the spectral parameters of melampodin-B were frequently used in the structural considerations of the other related compounds, the following summary of the physical and spectral data of melampodin-B was considered appropriate.

Melampodin-B (56), $C_{17}H_{18}O_7$, mp 226-228° (dec.), represented the minor component in one population of M. leucanthum but was the major, most polar, constituent in M. cinereum and M. argophyllum. The ir spectrum of 56 shows absorptions typical of an α,β-unsaturated γ-lactone (1780 cm^{-1}) and signals at 3420 and 1665 cm^{-1} are indicative of a hydroxyl group, and double bonds, respectively.



56



56a

The structure 56, however, was mainly deduced by correlating the 25.2MHz ^{13}Cmr and 300MHz pmr spectral data. The mass spectral patterns as well as the physical data of a number of derivatives of 56 corroborated these interpretations.

Carbon-13 NMR Data of Melampodin-B

The ^{13}Cmr spectrum of 56 was obtained under proton noise decoupled (PND) and single frequency off center decoupled (SFOCD) conditions. A PND spectrum of 56 indicated that the compound contains 17 carbon atoms; therefore, $C_{17}H_{18}O_7$ is the only empirical formula in agreement with both the ^{13}Cmr spectral data and the elemental analysis of 56. The residual splittings in the SFOCD

TABLE 1

Carbon-13 Parameters of Melampodin-B (56)

Skeletal Type	Chemical Shift	Assignment
	173.0 s	C-14
-C(C=O)-O	170.4 s	C-1'
	169.9 s	C-12
	148.7 s	C-10
$(C)_2$-C=	136.6 s	C-4
	131.1 s	C-11
	79.7 d	C-8
$(C)_2$-CH-O	73.9 d	C-6
	68.6 d	C-1
	154.2 d	C-9
C-HC=		
	122.9 d	C-5
	28.7 tr	C-3
C-CH_2-C		
	23.9 tr	C-2
H_2C=	123.0 tr	C-13
C-CH_2-O	65.3 tr	C-15
$(C)_2$-CH-C	50.3 d	C-7
-CH_3	21.7 q	C-2'

The spectrum was determined on a Varian XL-100-15 spectrometer operating Fourier transform mode with proton decoupling. The chemical shifts are relative to internal TMS and are recorded in ppm relative to TMS. PND, proton noise-decoupled spectrum; SFOCD, single-frequency off-center decoupled spectrum. The number of lines in the SFOCD spectrum are designated as follows: s, singlet; d, doublet; tr, triplet; q, quartet. Spectrum was obtained in pyridine-d_5.

spectrum of the compound indicate the number of hydrogen atoms attached to a particular carbon atom. Thus, a singlet signifies that the carbon has no attached hydrogens, a doublet indicates one, a triplet two, and a quartet three hydrogens at a carbon atom. Therefore, using both the chemical shift values (δ) and the pattern of a particular signal, it was determined that melampodin-B contains the following skeletal units:[69] a) three -C(C=O)-O, b) three $(C)_2$-C=, c) three $(C)_2$-CH-O, d) two C-HC=, e) two C-CH_2-C, f) one H_2C=, g) one C-CH_2-O, h) one $(C)_2$-CH-C, and i) one -CH_3 group (Table 1). Initial assignments of a number of chemical shifts for the respective carbon atoms of melampodin-B were made by comparison with the ^{13}Cmr spectral data of melampodin-A.[71]

PMR Spectral Data of Melampodin-B

The correlation of the ^{13}Cmr spectral data with the pmr spectral parameters of 56 (Figure 2, Table 2), which included extensive double resonance experiments, provided substantial evidence for the assigned structure of melampodin-B (56). The 300MHz spectrum of 56 in pyridine-d_5 exhibits doublets at δ6.47 and δ5.93 and a one proton multiplet at δ3.56. These signals are characteristic of α,β-unsaturated sesquiterpene γ-lactones. Upon irradiation of the signal at δ3.56 (H-7) both doublets at δ5.93 (H-13a) and δ6.47 (H-13b) collapsed to singlets while the triplet at δ4.99 (H-6) simplified to a broadened doublet and the broadened singlet at δ5.95 (H-8) sharpened to a singlet. Further decoupling experiments showed that the broadened doublet at δ5.99 (H-5) was coupled to

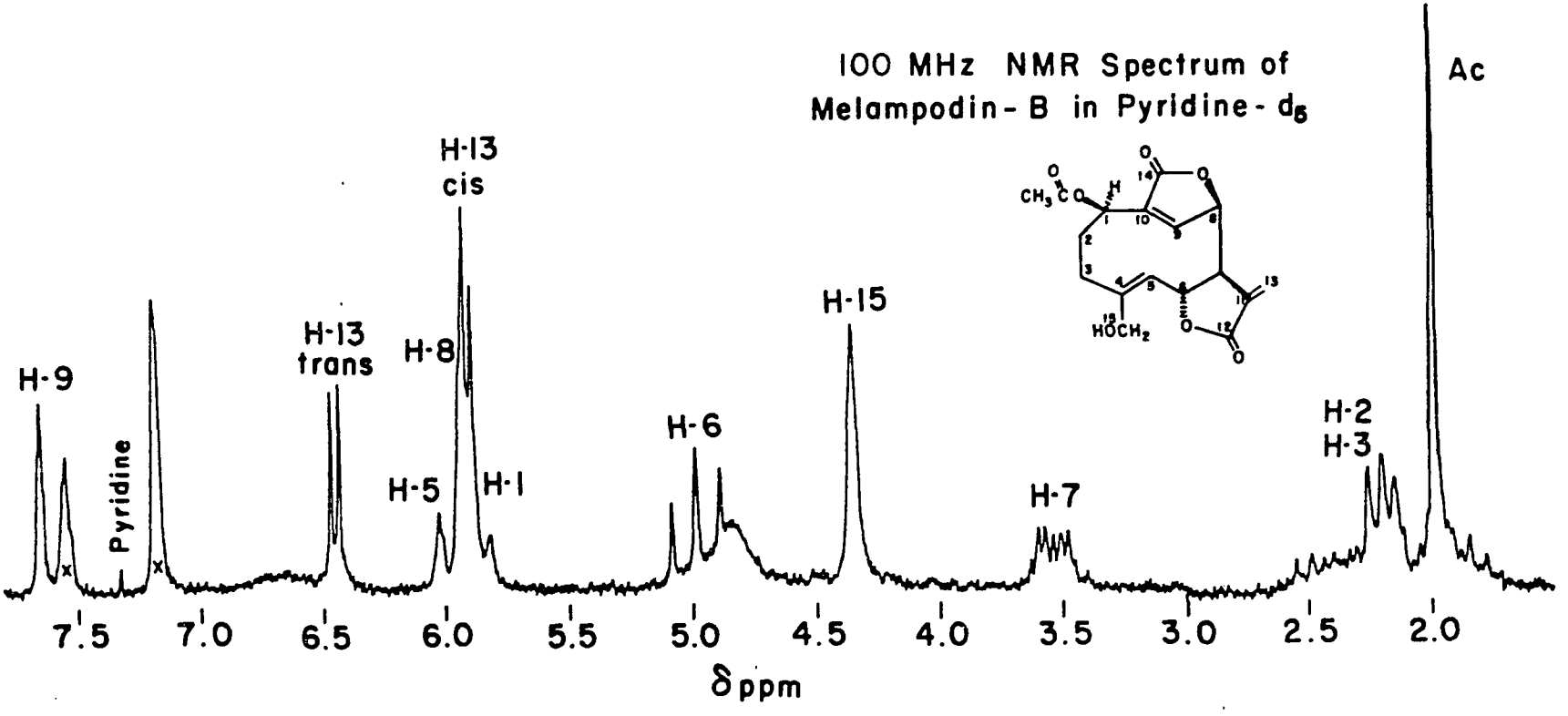

100 MHz NMR Spectrum of
Melampodin-B in Pyridine-d_5
Ac
H-13
cis
H-13
trans
H-8
H-15
H-9
Pyridine
H-5
H-1
H-6
H-7
H-2
H-3
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
δ ppm

FIGURE 2

TABLE 2

PMR Parameters of Melampodin-B (56)

Assignment	56[c,e]	56[b,f]
H-1	5.96 brdd (6.5, 6.5)	5.53 m
H-2a	1.92 m	x
H-2b	2.45 m	x
H-3	2.20 m	x
H-5	5.99 brd (10.0)	5.59 brd (10.0)
H-6	4.99 tr (10.0)	4.58 tr (10.0)
H-7	3.56 brdd (10.0, 3.5, 3.0)	3.46 m
H-8	5.95 brs	5.88 brs
H-9	7.63 brs	7.56 dd (1.0, 1.0)
H-13a	5.93 d (3.0)	5.93 d (3.0)
H-13b	6.47 d (3.5)	6.27 d (3.5)
H-15	a) 4.34 brd (15.0) b) 4.40 brd (15.0)	4.10 brs
Acetyl	2.02 s	2.03 s
-OH	6.60	x

a = $CDCl_3$, b = acetone-d_6, c = pyridine-d_5, e = 300MHz spectrum, f = 100MHz spectrum, Chemical shifts are in ppm (δ) relative to TMS as an internal standard. The following symbols are used to describe the respective signals: s, singlet; d, doublet; tr, triplet; q, quartet: p, pentet; h, heptet; x, obscured by overlapping signals; m, multiplet; Coupling constants in cps.

NOTE: Following NMR Tables have used the same symbols for describing the various spectral parameters.

the triplet at δ4.99 (H-6) and that the broadened singlet at δ5.95 (H-8) was coupled to the broadened singlet at δ7.63 (H-9). Acetylation of 56 caused a significant downfield shift of the broadened doublets at δ4.34 and δ4.40 (two diastereotopic C-15 methylene protons) while oxidation of 56 with Sarett's reagent resulted in a loss of these absorptions and the appearance of a signal at δ9.49 (typical of an aldehyde proton). The above data together with the observation that the oxidation of 56 caused the H-5 signal at δ5.99 to shift downfield to δ6.64 indicated the presence of a β-hydrogen in an α,β-unsaturated aldehyde in 58; this necessitated the presence of a primary, allylic alcohol function in 56.

In addition, the presence of a methyl singlet at δ2.02, typical of an acetate group, and the absence of any other resonances typical for methyl or aldehyde protons suggested that the C-4 and C-10 methyl functions in melampodin-B must be oxidized to alcohol and/or carboxylic acid functions. The structural data described above strongly suggested that 56 is a germacranolide-type sesquiterpene dilactone which contains one primary allylic alcohol and one acetate group. Since the germacranolides have a C_2-C_7 axis of symmetry, two structures are possible (given the above data) for melampodin-B. Subsequent decoupling experiments on the signals at δ1.92, δ2.45, (H-1a and H-1b), δ2.22 (H-2), δ5.96 (H-8), and δ7.63 (H-9) indicated that the acetoxy function is attached at C-1 (not C-3) and that the C-8 lactonic proton signal is at δ5.96.

The stereochemistry of melampodin-B was mainly deciphered by using the coupling constants of the C-5, C-6, C-7, and C-8

protons together with biogenetic considerations.[69] Additional stereochemical evidence for melampodin-B has been found in the present study and will be discussed in the following sections. Figure 3 illustrates the final structure of melampodin-B with its corresponding nmr parameters.

Mass Spectral Data of Melampodin-B

The reoccurance of a number of significant peaks in the mass spectra of melampodin-B, its derivatives, and analogs has proved to be very helpful in the structure elucidation of the germacranolide dilactones and we have frequently used them as diagnostic markers for newly isolated compounds (Schemes XI, XIII, and XVI). Due to the lack of isotopically labeled compounds final proof for the following mass spectral interpretations was not available. However, the suggested fragmentation patterns are quite reasonable and well documented in the literature. Intense peaks at m/e 274, 256, and 228 were generally observed in the melampodin-B type compounds. The mass spectrum of melampodin-B lacks a parent peak even under low voltage (20 MeV) conditions, but shows intense peaks at m/e 274, 256 (base peak), and 228. In contrast, melampodin-B acetate (57) gives a parent peak at m/e 376 and a peak at m/e 333 (M^+-43) indicating the loss of an acylium ion (CH_3CO^+) from the parent molecule. The fragment corresponding to m/e 274 (M^+ - 102) could be formed by a sequential or simultaneous loss of CH_3COOH (60 m.u.) and CH_2=C=O (42 m.u.). The loss of acetic acid by a McLafferty rearrangement[72], which occurs in acetate derivatives with hydrogen

NMR Spectral Parameters of Melampodin-B

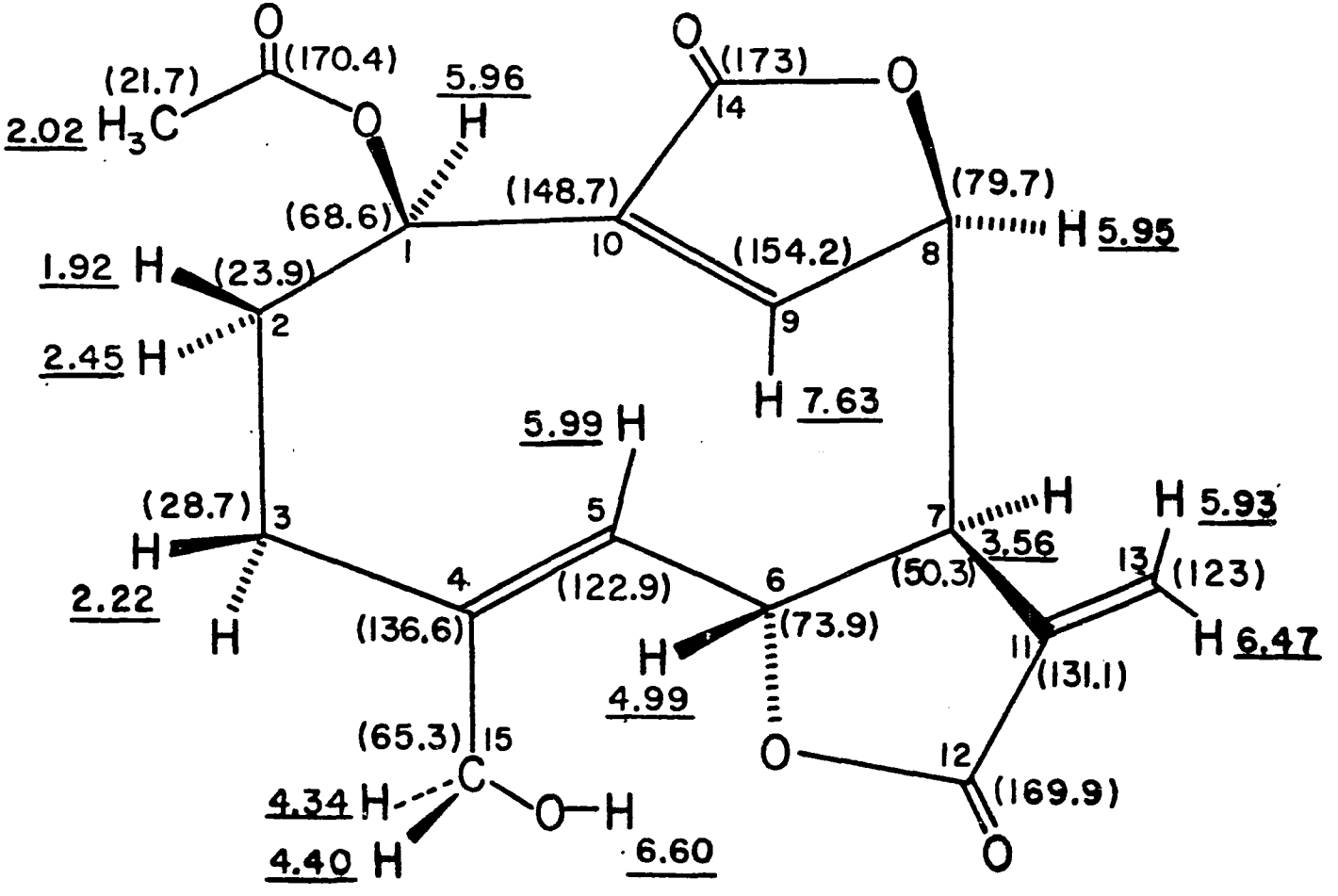
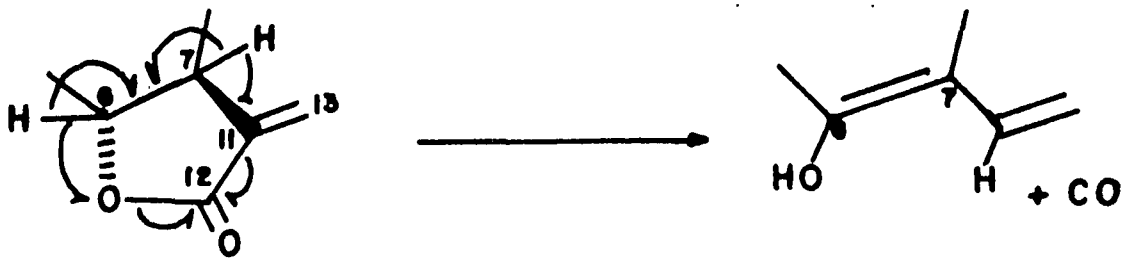


FIGURE 3

atoms β to the carbon atom to which the acetoxy group is attached, is well documented. The lack of a β-hydrogen allows the acetate group attached to C-15 in 57 only to form the neutral fragment, ketene (42 m.u.) and an ionized alcohol which gives rise to the peak at m/e 256 by the loss of H_2O (18 m.u.). The intense peak at m/e 228 in 56 and 57 could be due to the loss of CO (28 m.u.) from the fragment m/e 256 as illustrated by the following fragmentation:

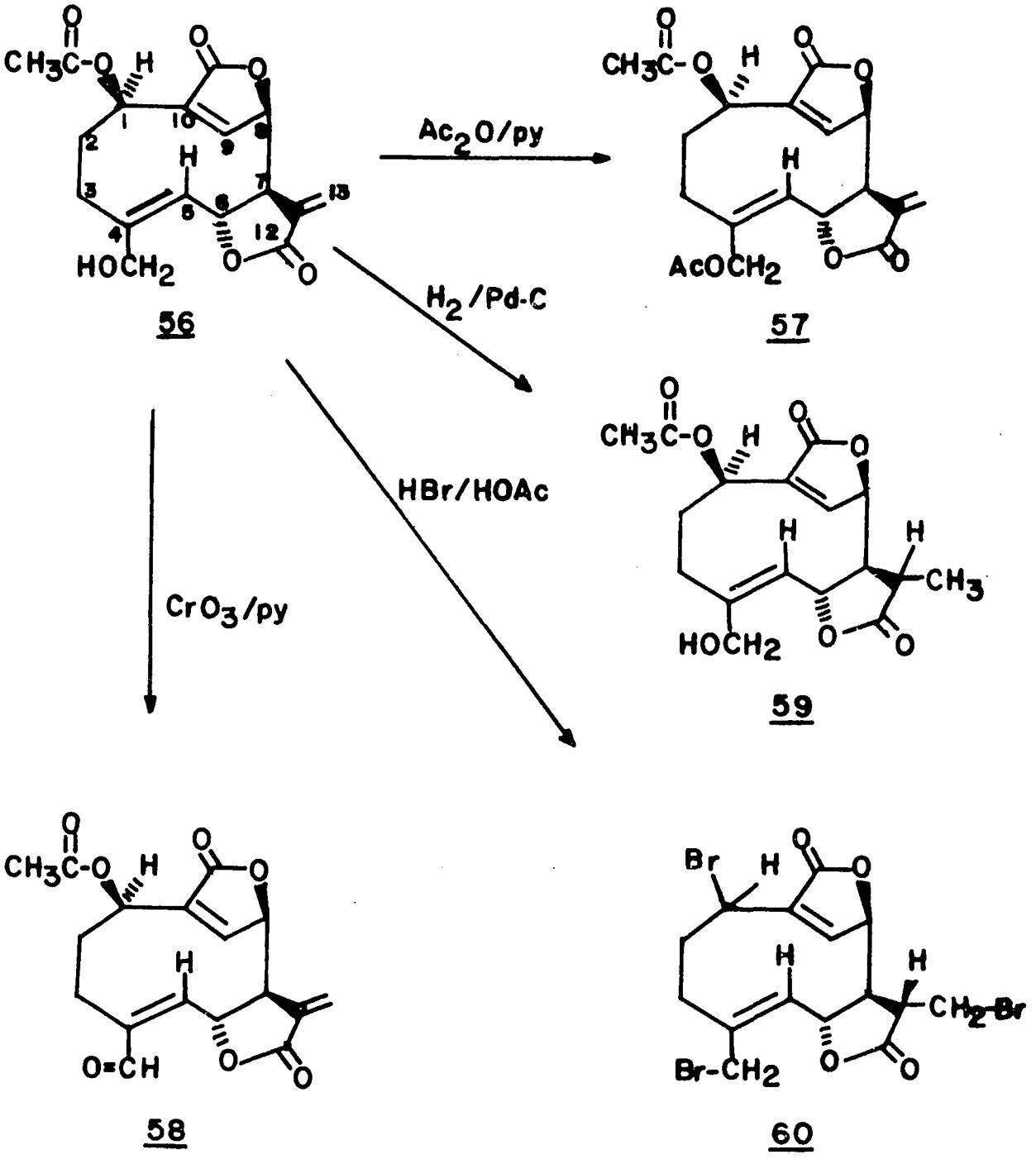


The loss of CO from γ-lactones appears to be common in these types of compounds.[73]

Melampodin-B Derivatives

Melampodin-B was converted into its acetate (57), aldehyde (58), and the 11(13)-dihydro derivative (59) primarily to change its pmr parameters and to correlate the mass spectral fragmentation patterns of these compounds. In addition, the correspondingly different elemental analysis of each derivative confirmed $C_{17}H_{18}O_7$ as the correct empirical formula of melampodin-B. In general, due to changes in solubility of these new compounds different solvents, and therefore different techniques (i.e. NOE, spin-decoupling, shift

MELAMPODIN-B DERIVATIVES



SCHEME X

TABLE 3

PMR Parameters of Melampodin-B Derivatives

Assignment	57[c]	58[c]	59[b]	60[b]
H-1	5.84 m	5.52 m	5.61 m	4.82 dd (12.0, 5.0)
H-5	5.69 d (10.0)	6.64 d (10.0)	5.50 brd (10.0)	5.67 d (10.0)
H-6	4.93 tr (10.0)	4.81 tr (10.0)	4.91 m	4.47 tr (10.0)
H-7	3.52 m	3.82 m	2.95 m	3.09 m
H-8	5.89 brs	5.96 brs	5.60 brs	5.72 brs
H-9	7.58 d (1.0)	7.71 brd (1.0)	7.33 s	7.90 d (2.0)
H-13a	5.89 d (3.0)	6.05 d (3.0)	1.40 d	3.94 d
H-13b	6.41 d (3.5)	6.36 d (3.5)	(8.0)	
H-15	4.69 d (1.0)	9.48 d (1.5)	4.17 d (6.0)	a) 4.10 dd (1.0, 1.0) b) 4.27 dd (1.0, 1.0)
Acetyl	2.02 s	2.10 s	2.08 s	
H-11			4.91 m	3.50 ddd (11.0, 4.0, 4.0)
-OH			3.94 dd (1.0,5.0)	

b = acetone-d_6; c = pyridine-d_5. All spectra are 100MHz.

reagent studies) can be employed in nmr experiments. The structural feature most easily recognized as a result of the conversion of 56 into 57 and 58 was the position and type of hydroxyl substitution. As previously mentioned, the broadened doublets at δ4.34 and δ4.40 in 56 appear downfield at δ4.69 in 57 as a broadened doublet indicating the presence of a hydroxyl group. The downfield shift of this two proton signal in 57 suggested the presence of a primary alcohol in 56. Oxidation of 56 with Sarett's reagent to give 58 confirmed this hypothesis. The presence of an aldehydic proton at δ9.49 and a downfield shift of the H-5 doublet at δ5.99 in 56 to δ6.60 in 58 also indicated that 56 is an allylic alcohol. The chemical shift of the H-5 doublet is typical for a β-hydrogen in an α,β-unsaturated aldehyde. The observed allylic coupling between H-5 and H-15 in 58 also corroborated the assignment of the C-4(5) double bond with a hydroxyl group at C-15 in 56 and a C-15 aldehyde function in 58.

The remaining peaks in the pmr spectra of 57 and 58 closely resemble the corresponding peaks in 56, both in chemical shift and coupling constant values (Table 3). On the basis of these observations it was concluded that the remaining parts of the molecule were not affected in the chemical conversion of 56 into 57 and 58. Since there are no new chiral centers formed or transformed in the formation of 57 and 58, the stereochemistry of these derivatives was assigned by applying the same biogenetic and pmr considerations that were employed for melampodin-B.

The hydrogenation of melampodin-B was undertaken for two reasons: 1) to gain an understanding of the reactivity and

stereochemical consequences involved in the hydrogenation of the different double bonds in 56; 2) the hydrogenation product(s) could possibly be used to correlate melampodin-B with its biogenetic 4(5)-dihydroproduct, another new sesquiterpene lactone which will be discussed later. Brief hydrogenation of 56 over 10% Pd/C provided 11(13)-dihydromelampodin-B (59) indicating that the more highly substituted C-4(5) and C-10(9) double bonds are sterically less accessible to saturation. Hydrogenation with the more vigorous PtO_2 catalyst removed all three double bonds in 56. The stereochemistry of 59 could not be accurately assigned since a mixture of closely related isomers, possibly the C-11 epimers, seemed to be the dihydroproducts.

Melampodin-B was transformed into the tribromide 60 using a saturated solution of HBr in glacial acetic acid. Under these conditions either a S_N1 or S_N2 reaction could have occurred involving the allylic acetoxy function at C-1 and the allylic alcohol at C-15. An acid-catalyzed Michael addition must have taken place at C-13 of the α,β-unsaturated lactone. In the case of the substitution at C-15, the resulting stereochemistry is not relevant, while at C-1, the reaction could have occurred with retention or inversion of configuration at the chiral center in 56. The observed coupling constants ($J_{1,2a}$= 12.0Hz., $J_{1,2b}$= 5.0Hz.) for the H-1 doublet of doublets centered at δ4.83 can only be explained if one of the C-2 protons (H-2a) is anti-periplanar to the C-1 proton while the torsional angle between the other C-2 proton (H-2b) and H-1 is approximately 60°. Unfortunately, stereomodels indicated that these requirements can be met with H-1 in either an α or β-orientation

depending upon the conformation around the carbon atoms 1, 2, and 3 in 60. Thus the absolute stereochemistry at C-1 in 60 could not be determined by spectral methods. The multiplet at δ3.50 ($J_{7,11}$-11.0 Hz., $J_{11,13a}$= $J_{11,13b}$= 4.0 Hz.) in 60 was assigned to H-11. The large coupling constant ($J_{7,11}$= 11.0 Hz.) is typical of two protons in an anti-periplanar orientation; therefore, because H-7 was assumed to be α-oriented on biogenetic grounds, H-11 must be β-oriented. In 60, the two diastereotopic protons at C-15 are non-equivalent due to the probable steric interactions between the bromine at C-15 and neighboring groups which must prevent free rotation about the C_4-C_{15} axis. Thus, two H-15 signals which are doublet of doublets, geminally as well as allylically coupled, appear at δ4.10 and δ4.27. In contrast, there must be free rotation about the C_{11}-C_{13} axis since the equivalent H-13a and H-13b appear as a sharp doublet at δ3.94. Spin-spin decoupling experiments involving the signals of H-1, H-5, H-6, H-7, H-8, H-9, H-11, H-13, and H-15 verified the structural assigmments of 60 (Figure 4). The similarity of the coupling constants (H-5, H-6, H-7, H-8, and H-9) of 60 and 56 seemed to be an expression of stereochemical and conformational similarity between 60 and melampodin-B (56).

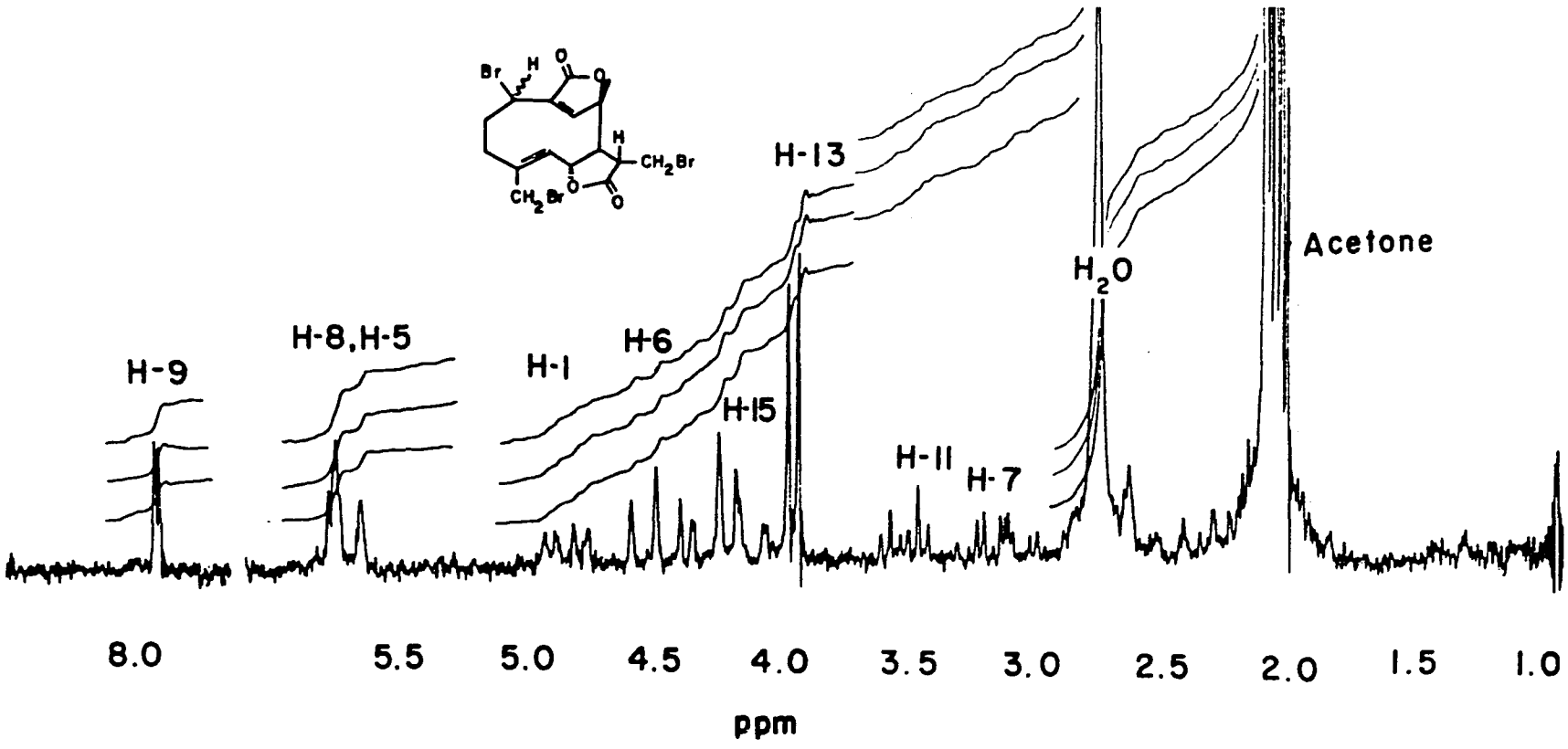
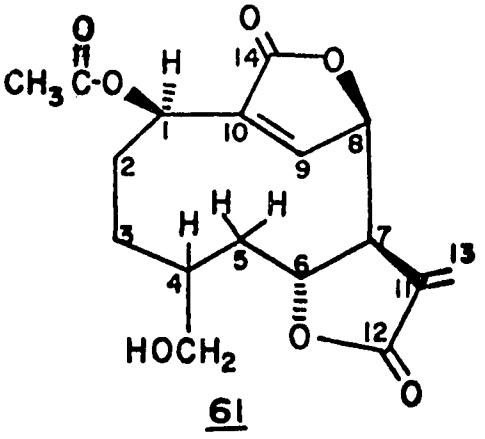

H-9
H-8,H-5
H-1
H-6
H-13
H-15
H-11
H-7
H2O
Acetone
8.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

FIGURE 4

4(5)-Dihydromelampodin-B

4(5)-Dihydromelampodin-B (61), $C_{17}H_{20}O_7$, mp. 204-205°, is a colorless crystalline germacranolide dilactone which has been isolated from only a few populations of M. cinereum. It shows ir absorptions (nujol) at 3400, 1785, 1750, and 1665 cm^{-1} values, which are quite similar to absorptions in melampodin-B. Compound 61 must contain a hydroxyl function (3400 cm^{-1}), a strained γ-lactone (1785 cm^{-1}), and α,β-unsaturated ester (1750 cm^{-1}), and double bonds (1665 cm^{-1}) which are also present in 56.[70] The structure of 4(5)-



61

dihydromelampodin-B (61) was mainly elucidated by correlating its 100MHz pmr parameters and mass spectral fragmentation patterns with those of melampodin-B.

In the 100MHz pmr spectrum of the new compound, doublets at δ5.92 and δ6.45 and a multiplet at δ3.43 signified that this compound represents an α,β-unsaturated γ-lactone (Figure 5, Table 4). Irradiation of the multiplet at δ3.43 (H-7) collapsed the doublets at δ5.92 and δ6.45 (H-13a and H-13b), simplified the multiplet at

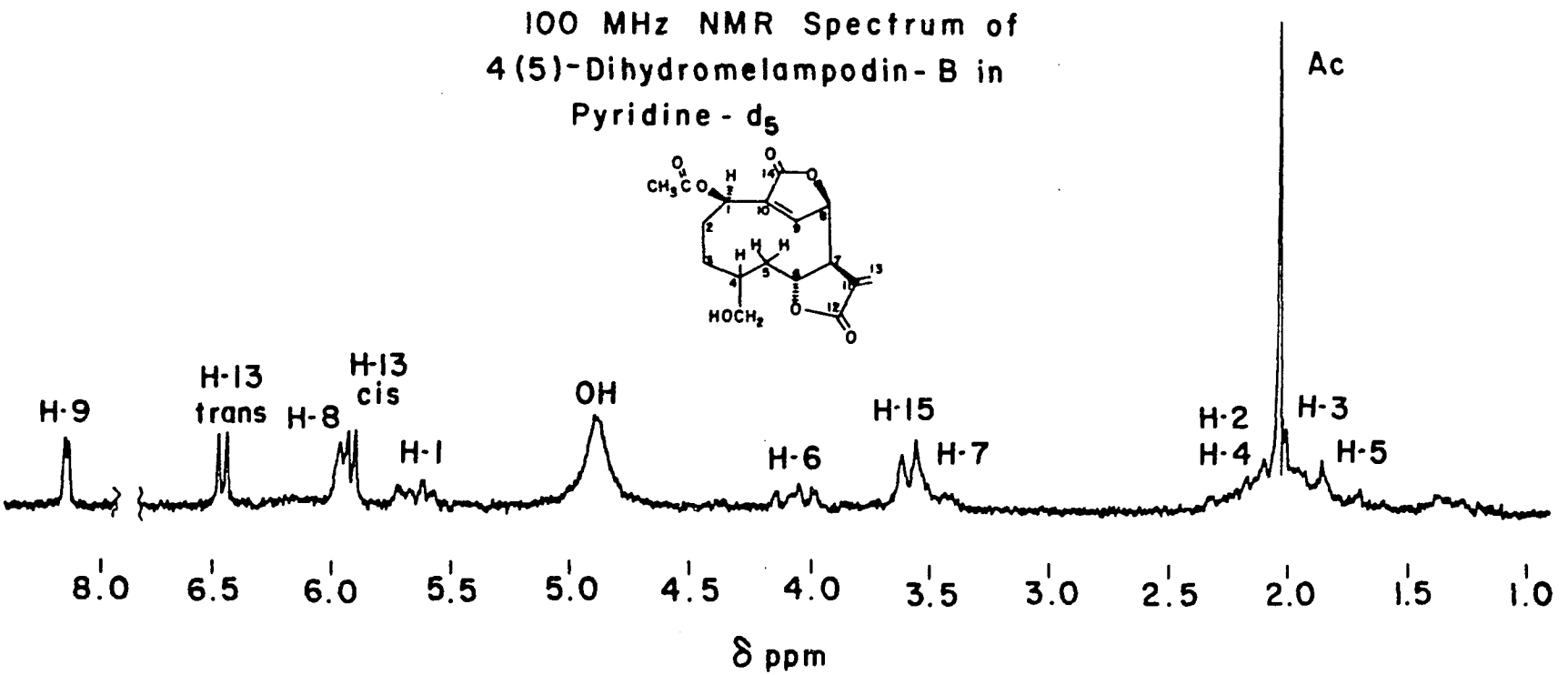

100 MHz NMR Spectrum of
4(5)-Dihydromelampodin-B in
Pyridine - d_5
Ac
H-9
H-13
trans
H-8
H-13
cis
H-1
OH
H-6
H-15
H-7
H-2
H-4
H-3
H-5
8.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
δ ppm

FIGURE 5

TABLE 4

PMR Parameters of 4(5)-Dihydromelampodin-B (61) and Derivatives

Assignment	61[b]	61[c]	62[a]	63[b]
H-1	5.30 m	5.65 m	5.37 m	5.39 ddd (5.5,5.5,1.0)
H-6	3.50 m	4.05 m	3.76 m	3.97 m
H-7	3.37 m	3.43 m	3.13 m	3.35 m
H-8	5.92 brs	5.96 brs	5.61 brs	5.58 brs
H-9	8.03 d (1.0)	8.13 d (1.0)	7.51 d (1.5)	7.84 dd (1.0,1.0)
H-13a	6.00 d (3.0)	5.91 d (3.0)	5.82 d (3.0)	
H-13b	6.31 d (3.5)	6.45 d (3.5)	6.44 d (3.5)	1.33 d (8.0)
H-15	3.70 m	3.58 d (6.0)	3.87 d (7.0)	3.37 d (6.0)
Acetyl	2.02 s	2.03 s	2.06 s	2.03 s
H-11				2.93 m
-OH				3.71 tr (5.5)

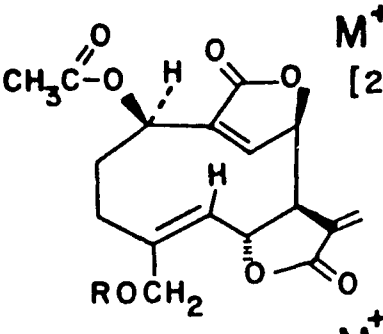
a = $CDCl_3$; b = acetone-d_6; c = pyridine-d_5. Spectra are 100MHz.

δ4.05 (H-6), and sharpened the broadened singlet at δ5.96 (H-8). Irradiation of the H-8 signal simplified the multiplet at δ3.43 (H-7) and collapsed the downfield doublet at δ8.03 (H-9) to a broadened singlet. At this point, the gross similarities between the pmr data of 4(5)-dihydromelampodin-B (61) and melampodin-B (56) were apparent. The major pmr spectral differences between 56 and 61 were observed in the C-6 and C-15 proton signals. In melampodin-B, the C-6 lactonic proton signal appears as a sharp triplet at δ4.99 while in 61 the H-6 signal represents a complex multiplet at δ4.03. This implied that more than one proton is attached to C-5. Further evidence that 61 is a dihydroderivative of melampodin-B was given by a two proton doublet at δ3.58 which suggested the presence of a methylene group (two C-15 protons) coupled to a proton at C-4. Indeed, double resonance experiments on 4(5)-dihydromelampodin-B acetate (62) showed that irradiation at δ3.88 (C-15 protons) affected the envelope at about δ2.00, while irradiation at δ2.00 (H-4) caused the doublet at δ3.88 to collapse. This indicated that 61 and 62 contain a C-4 proton that is not present in melampodin-B.

Mass Spectral Data of 4(5)-Dihydromelampodin-B and Derivatives

Further evidence concerning the structure of 61 came from the correlation of the mass spectral fragmentation patterns of melampodin-B (56) and its acetate (57) with the fragmentation patterns of 61 and 62 (Scheme XI). Both 61 and 62 give parent peaks at m/e M^+336 and M^+378, respectively; therefore, it was immediately apparent that the major peaks at m/e 276, 258, and 230 in 61 and 62

MASS SPECTRAL DATA of MELAMPODIN B-TYPE COMPOUNDS(A)



R = H
R = Ac

M^+ 376 − 43 ($CH_3C\equiv\overset{+}{O}$) → 333 [26.5]
[21.8]

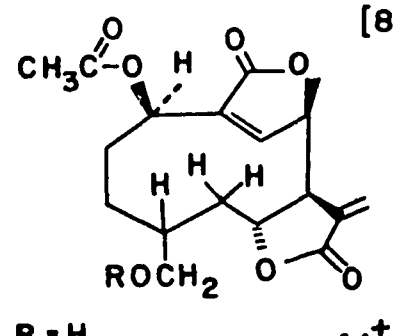
−59 (CH_3COO)

274 [97.0] [53.5] −18 (H_2O) → 256 [100] [100]

−28 (CO)

228 [93] [96.5]

M^+ 334 − 60 (CH_3COOH)



R = H
R = Ac

M^+ 378 − 43 ($CH_3C\equiv\overset{+}{O}$) → 335 [18.8]
[81.3]

−59 (CH_3COO)

276 [70.0] [59.6] −18 (H_2O) → 258 [100] [100]

−28 (CO)

230 [81.3] [202]

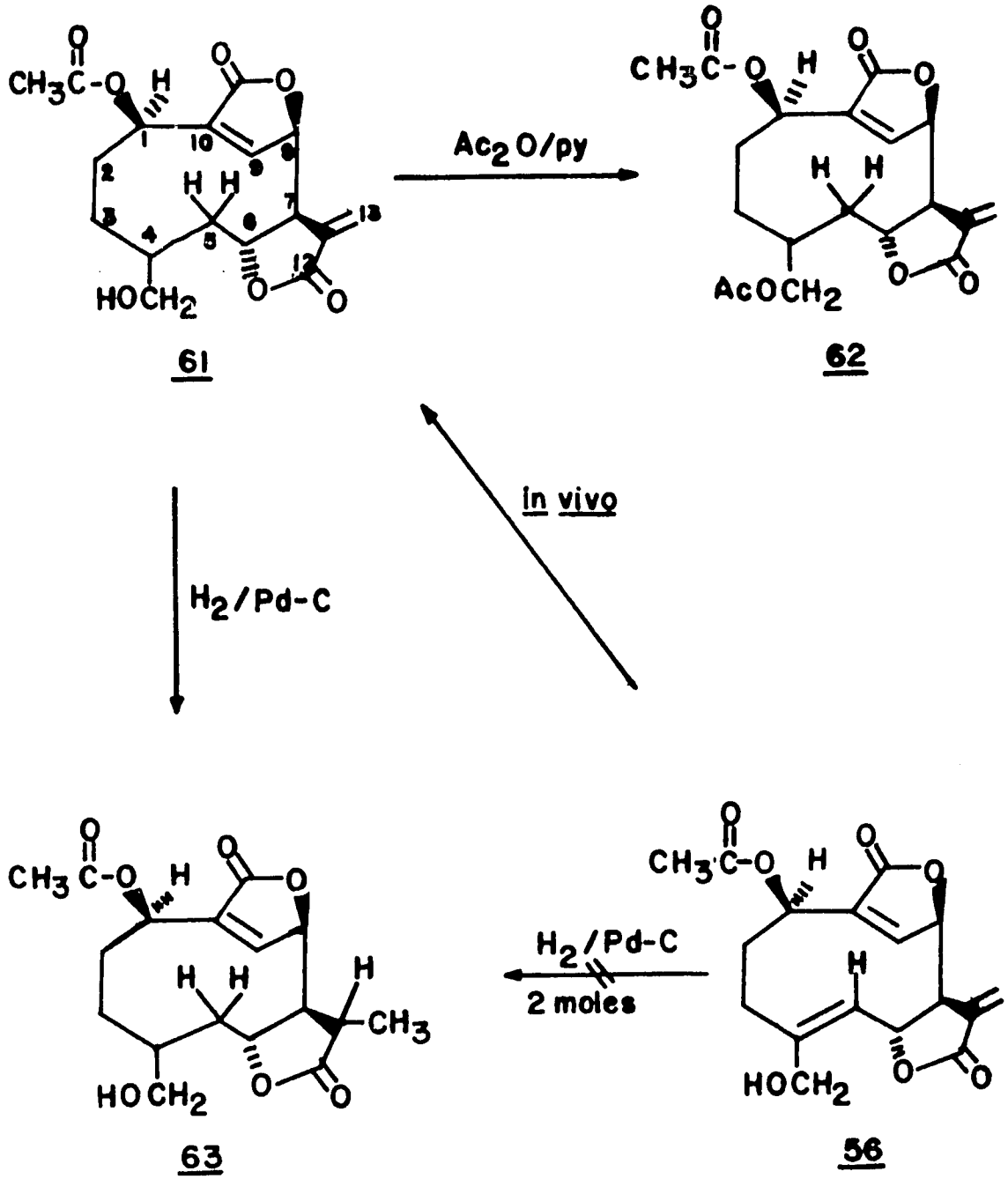
M^+ 336 − 60 (CH_3COOH)
[71.5]

SCHEME XI

differed from those in 56 and 57 (i.e. m/e 274, 256, and 228) by two mass units. Furthermore, the difference of two mass units has to be situated in the medium rings of 61 and 62. The pmr spectra of 61 and 62 contain the signals typical of exocyclic methylene doublets (C-13 protons) and the downfield doublet typical of the β-hydrogen at C-9 of the α,β-unsaturated lactone. Therefore, the absence of the C-4(5) double bond in 61 must represent the structural difference between melampodin-B and 4(5)-dihydromelampodin-B.

The assignments of the peaks at m/e 276, 258, and 230 involved the same arguments which were used in the assignment of the melampodin-B fragmentation patterns. That is, 4(5)-dihydromelampodin-B acetate (62) gives a parent peak at m/e 378 and a peak at m/e 335 (M^+ - 43) indicating the loss of an acylium ion (CH_3CO^+) from the parent molecule. The fragment corresponding to m/e 276 (M^+ - 102) could be formed by a sequential or simultaneous loss of CH_3COOH (60 m.u.) and $CH_2=C=O$ (42 m.u.). The loss of acetic acid from acetate derivatives (both 61 and 62) with hydrogens β to the carbon atom carrying the acetoxy group occurs via a McLafferty rearrangement.[71] The lack of a β-hydrogen allows the acetoxy group at C-15 in 62 to form only the neutral fragment, ketene (42 m.u.), and an ionized alcohol which gives rise to the peak at m/e 258 by the loss of H_2O (18 m.u.). The intense peak at m/e 230 in 61 and 62 could be due to the loss of CO (28 m.u.) from the m/e 258 fragment (Scheme XI).

4(5)-DIHYDROMELAMPODIN-B DERIVATIVES



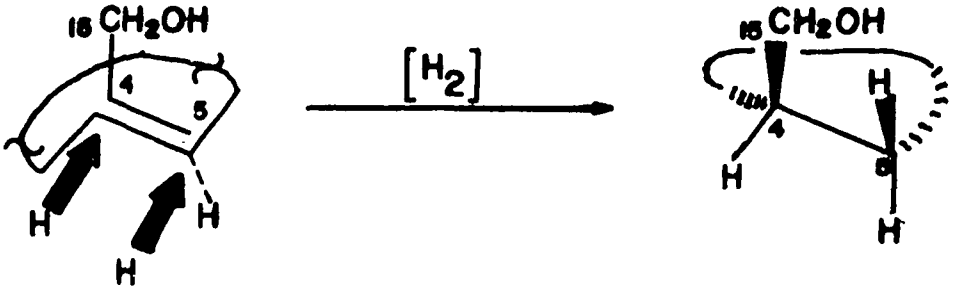
61 62 63 56

SCHEME XII

4(5)-Dihydromelampodin-B Derivatives

4(5)-Dihydromelampodin-B was converted into its acetate (62) and the 4(5),11(13)-tetrahydromelampodin-B (63) primarily to corroborate $C_{17}H_{20}O_7$ as its empirical formula. The previously mentioned mass spectral and pmr data of 62 gave substantial evidence for the correct structural assignments of 61. The anticipated formation of the tetrahydro-derivative 63 from both 61 and 56 by catalytic hydrogenation would have provided chemical evidence for the structure of 61. Unfortunately, catalytic hydrogenation of melampodin-B with 10% Pd/C gave only 11(13)-dihydromelampodin-B (59) which resisted a further uptake of hydrogen to give the tetrahydro-derivative 63, a necessary step for the correlation of 56 with 61 (Scheme XII).

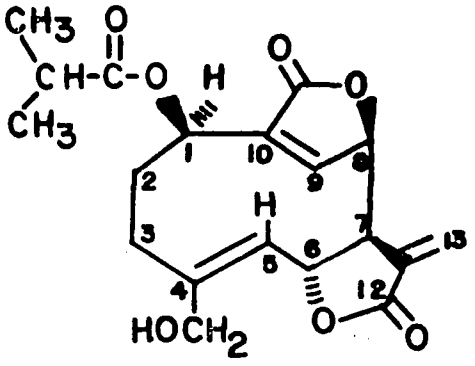
Melampodin-B (56) should represent the biogenetic precursor for the 4(5)-dihydro compound 61. Since 56 has a fixed conformation, the C-4(5) double bond is quasi-perpendicular to the medium ring and the C-15 group is oriented above the plane of the medium ring.[69] Consequently, a biogenetic reduction process via front-side attack should lead to a β-oriented C-15 group in 61 (following diagram).



Due to the absence of a double bond at C-4(5) in 61, the medium ring is more flexible and possibly exists in different conformations, in contrast to a fixed conformation in melampodin-B. On the basis of the above biosynthetic assumption the chiral centers at C-1, C-6, C-7, and C-8 in 61 should remain the same in the conversion of melampodin-B (56) to 4(5)-dihydromelampodin-B (61).

MELAMPODIN-C

Melampodin-C (64), $C_{19}H_{22}O_7$, mp 199-201°, co-occurred with melampodin-B in Melampodium argophyllum, a seldomly found plant in the mountains of northern Mexico. IR absorptions of 64 were indicative of a hydroxyl group (3500 cm^{-1}), a γ-lactone (1765 cm^{-1}, and an α,β-unsaturated ester (1720 cm^{-1}).[70] The structure of melampodin-C was inferred from the correlation of the pmr spectral



64

parameters with those of melampodin-B (56). The mass spectral fragmentation patterns of the two compounds showed close similarities between the medium ring systems of 64 and 56, and the conversion of both 64 and melampodin-B (56) to the tribromide 60 implied that the medium ring portions of 56 and 64 are identical.

The 100 MHz spectra of melampodin-C (Table 5, Figure 6) and of melampodin-B (Table 2, Figure 2) exhibit gross similarities. Instead of the acetyl methyl singlet at δ2.02 in 56, a pair of doublets appear at δ1.11 in 64 which integrate for six protons. An additional one-proton heptet at δ2.52 suggested that the C-1 side

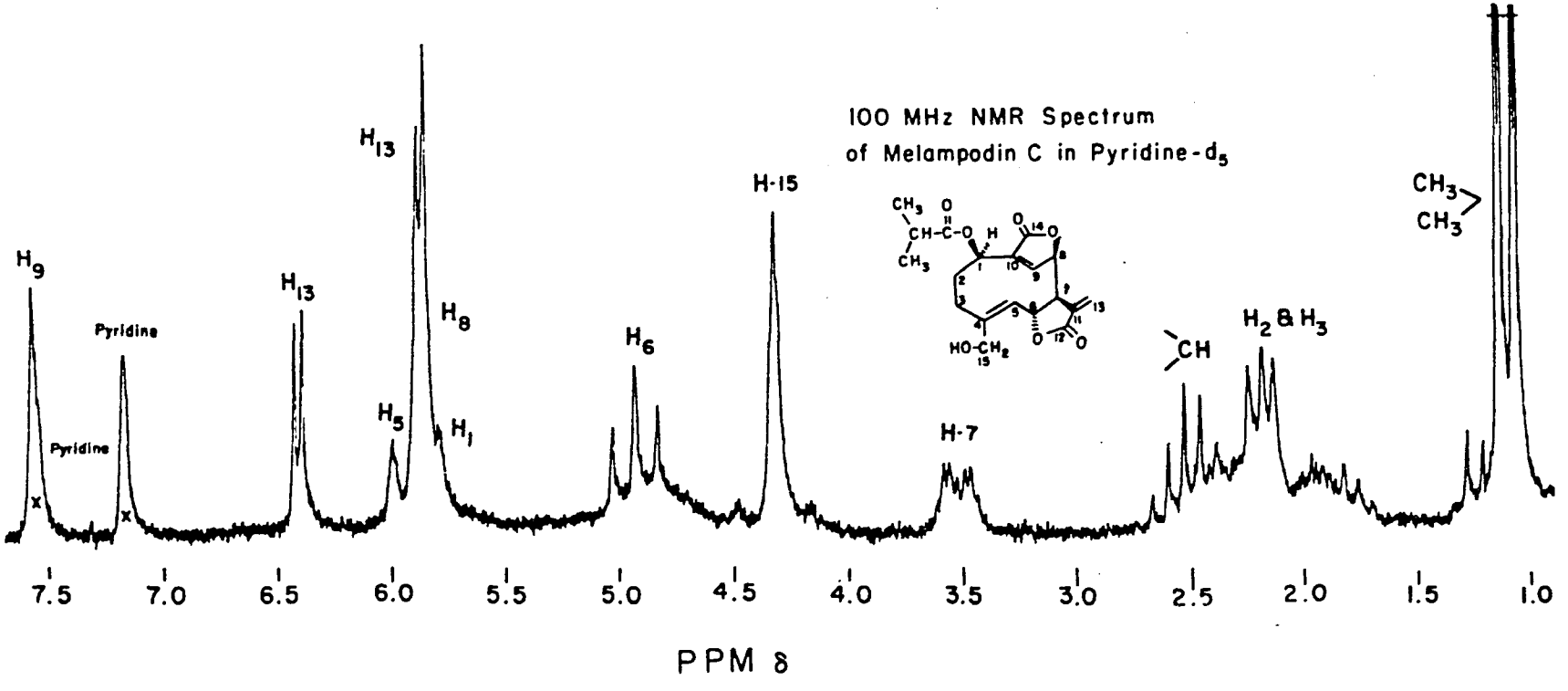

100 MHz NMR Spectrum
of Melampodin C in Pyridine-d5
H9
Pyridine
Pyridine
H13
H13
H5
H8
H1
H6
H-15
H-7
CH
H2 & H3
CH3
CH3
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
PPM δ

FIGURE 6

TABLE 5

PMR Parameters of Melampodin-C (64) and Derivatives

Assigmment	64[b]	64[c]	65[b]	66[b]
H-1	5.55 m	5.85 m	5.66 m	5.61 m
H-5	5.60 d (10.0)	5.95 d (10.0)	5.49 d (10.0)	6.40 d (10.0)
H-6	4.61 tr (10.0)	4.94 tr (10.0)	4.66 tr (10.0)	4.82 tr (10.0)
H-7	3.48 m	3.51 m	3.16 m	3.38m
H-8	5.86 brs	5.89 brs	5.58 brs	5.61 brs
H-9	7.54 d (1.5)	7.56 d (1.5)	7.14 d (1.5)	7.11 s
H-13a	5.92 d (3.0)	5.88 d (3.0)	5.80 (3.0)	5.90 d (3.0)
H-13b	6.27 d (3.5)	6.42 d (3.5)	6.43 d (3.5)	6.51 d (3.5)
H-15	4.14 d (1.0)	4.33 brs	4.62 d (1.5)	9.47 d (1.0)
3',4' Me	1.15 d (7.0)	1.11 d (7.0)	1.19 d (7.0)	1.14 d (7.0)
H-2'	2.58 ht (7.0)	2.52 ht (7.0)	2.53 ht (7.0)	2.50 ht (7.0)
Acetyl			2.11 s	

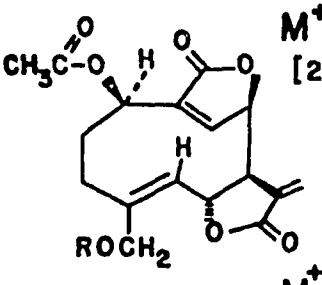
b = acetone-d_6; c = pyridine-d_5; Spectra are 100MHz.

chain in 64 contains an isopropyl group. Elemental analysis data substantiated $C_{19}H_{22}O_7$ as the empirical formula of melampodin-C; thus, the C-1 side chain must represent an isobutyrate group. Irradiation of the multiplet at δ3.51 (H-7) resulted in the following observations: a) the collapse of the doublet at δ5.88 (H-13a) and the doublet at δ6.42 (H-13b) to singlets; b) the sharpening of the signal at δ5.89 (H-8); c) the collapse of the triplet at δ4.94 (H-6) into a broadened doublet. Further double resonance experiments involving the heptet at δ2.52 and the two methyl doublets at δ1.11 confirmed the hypothesis that the C-1 side chain contains an isopropyl group. Double irradiations at the H-1, H-5, H-6, H-8, and H-9 signals substantiated the conclusion that the skeletal arrangement of the medium ring in melampodin-C is identical to melampodin-B.

Mass Spectral Data of Melampodin-C and Derivatives

The mass spectra of melampodin-C (64) and its acetate (65) contain the typical peaks at m/e 274, 256, and 228 in addition to their respective parent peaks at m/e M^+362 and M^+404. McLafferty rearrangement in 64 (M^+362) was hypothesized in order to account for the loss of the C-1 isobutyrate moiety (88 m.u.) and the resulting major fragment at m/e 274. In 65 (M^+404) the peak at m/e 361 (M^+-43) indicates the loss of an acylium ion (CH_3CO^+) from the parent molecule while the fragment corresponding to m/e 274 (M^+-130) could be formed by a sequential or simultaneous loss of $(CH_3)_2CHCOOH$ (88 m.u.) and $CH_2=C=O$ (42 m.u.). As before, the loss of isobutyric acid from 65 occurs via a McLafferty rearrangement

MASS SPECTRAL DATA of MELAMPODIN B-TYPE COMPOUNDS(B)



R = H
R = Ac

M^+ 376 [21.8] $-43(CH_3C\equiv\overset{+}{O})$ → 333 [26.5]

-59 (CH_3COO)

274 [97.0] [53.5] $-18(H_2O)$ → 256 [100] [100]

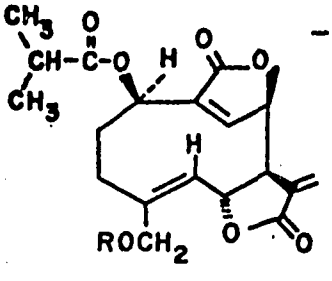
-28 (CO)

228 [93] [96.5]

M^+ 334 $-60(CH_3COOH)$

M^+ 404 [22.9] $-43(CH_3C\equiv\overset{+}{O})$ → 361 [10.4]

-87 $[(CH_3)_2CHCOO]$



R = H
R = Ac

-87 $[(CH_3)_2CHCOO]$

317 [60.4] $-43(CH_3C\overset{+}{O})$ − 274 [85.5] [145] $-18(H_2O)$ → 256 [100] [100]

-28 (CO)

M^+ 362 [5.0] $-88[(CH_3)_2CHCOOH]$

228 [77.0] [112]

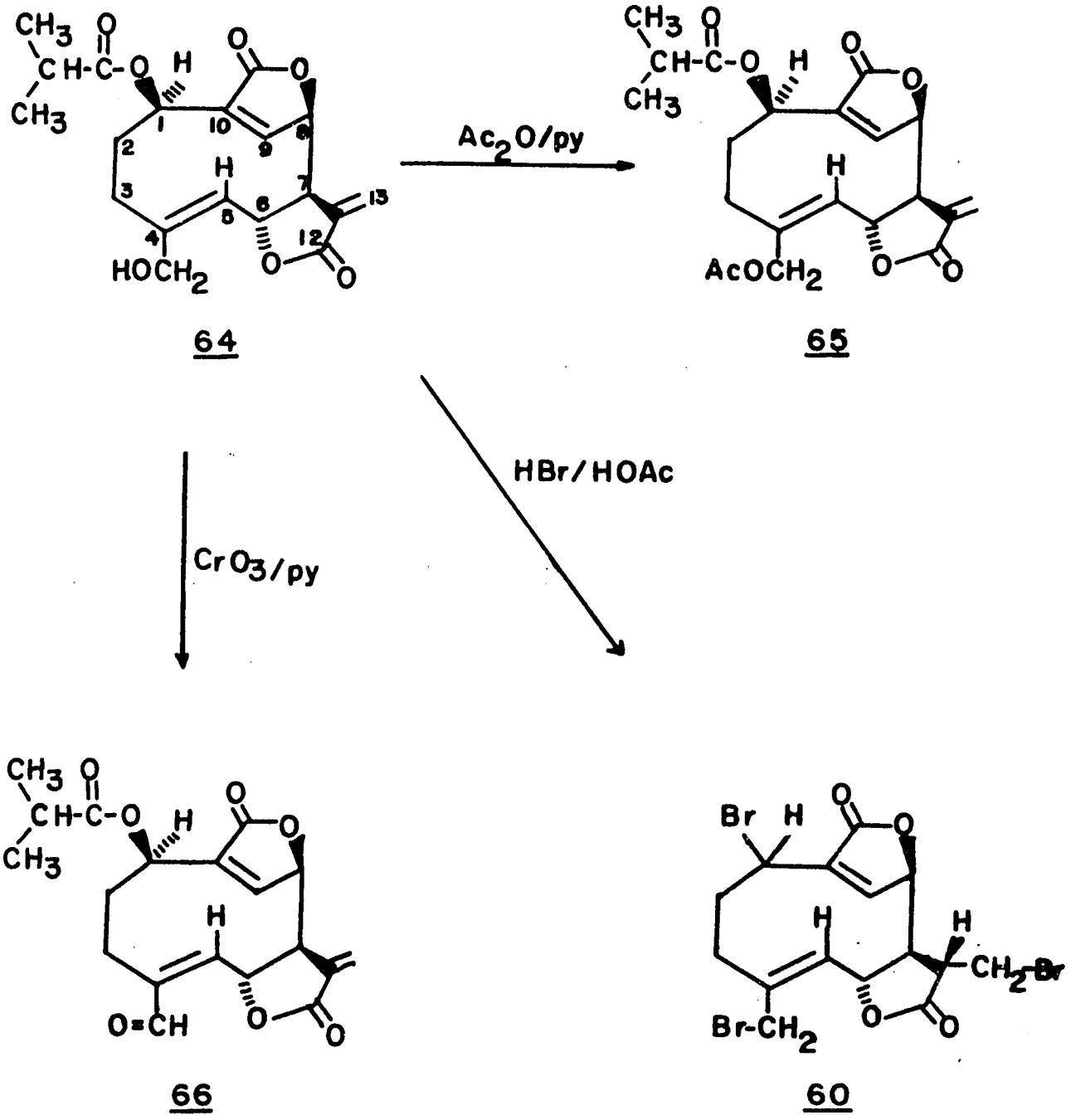
SCHEME XIII

since 65 has hydrogen atoms β to the carbon atom to which the isobutyrate group is attached. The lack of a β-hydrogen allows the acetate group attached to C-15 in 65 to form only the neutral fragment, ketene (42 m.u.) and an ionized alcohol which gives rise to the peak at m/e 256 by the loss of H_2O (18 m.u.). The intense peak at m/e 228 in 64 and 65 could result from the loss of CO (28 m.u.) from the fragment m/e 256. The correlation of melampodin-B with melampodin-C is shown in Scheme XIII.

Melampodin-C Derivatives

Melampodin-C was transformed into the acetate 65, the aldehyde 66, and the tribromide 60 (Scheme XIV); their elemental analyses confirmed $C_{19}H_{22}O_7$ as the empirical formula of 64. Derivatives 65 and 66 gave conclusive proof that melampodin-C contains a primary allylic alcohol at C-15 for the following reasons: a) the two-proton doublet which appeared at δ4.14 in 64 was shifted downfield to δ4.62 in 65; b) the acetate 65 contained one acetyl methyl signal at δ2.11; c) the doublet at δ5.60 (H-5) in 64 was found downfield at δ6.40 in 66; d) the H-15 signal in 64 was lost and a signal at δ9.47, typical of an aldehydic proton, was found in 66; e) the allylic coupling between H-5 and H-15 was observed in 66. The tribromo-compound (60) was formed from melampodin-C as well as from melampodin-B (56). This chemical conversion implied that both 56 and 64 have the same medium ring skeleton. It could be argued, however, that under the given reaction conditions (HBr/HOAc) a 7,6-lactone and a 7,8-lactone could, upon ring opening and

MELAMPODIN-C DERIVATIVES



64 65 66 60

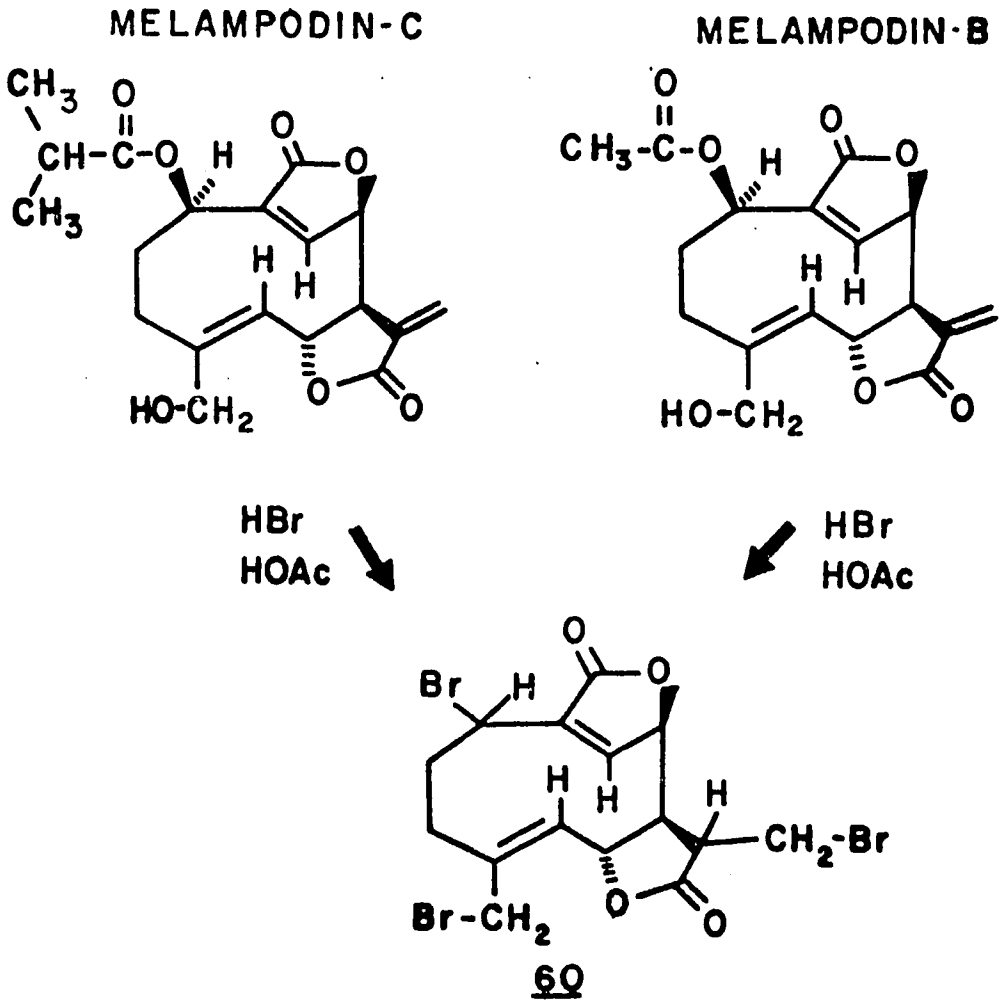
SCHEME XIV

relactonization, lead to the same tribromide 60. The close similarities of the pmr spectral data of the original compounds 56 and 64 rule out this possibility; that is, both 56 and 64 already represent 7,6-lactones. Hence, this transformation proved that the structural difference between melampodin-C and melampodin-B must be restricted to the side chain at C-1 (Scheme XV).

Stereochemistry of Melampodin-C and Derivatives

Two biogenetic assumptions were made in the stereochemical assignments of melampodin-C: 1) the H-7 has an α-orientation relative to the plane of the medium ring (as was found in the related melampodin-A, the absolute configuration of which is known[29,31]), and 2) the C-4(5) double bond has a trans-configuration. The proton spin-spin interactions ($J_{5,6}$= 10.00Hz; $J_{6,7}$= 10.0Hz; $J_{7,8}$= 2.5Hz; and $J_{8,9}$= 1.5Hz) suggested that the torsion angles between the respective protons are approximately, 180, 180, 80, and 80°. These spectral assignments are in full agreement with the skeletal arrangement as illustrated in 64. Inspection of stereomodels for the different isomeric possibilities showed that the only other skeletal arrangement of 64 which would give similar torsion angles for these respective protons contains a cis-C-4(5) double bond. Two observations excluded this possibility: 1) the compelling chemical and spectral evidence which implied identical skeletal arrangements for melampodin-C and melampodin-B; 2) the observed coupling constant between H-7 and H-8 ($J_{7,8}$= 2.5Hz) is too small for a torsional angle of approximately

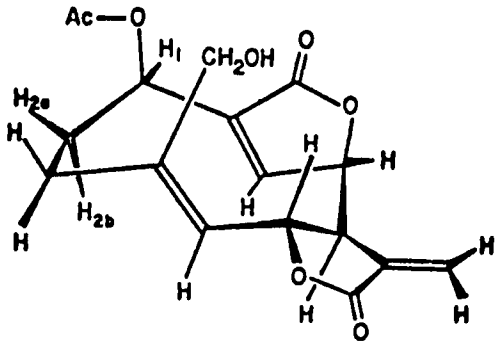
CORRELATION of MELAMPODIN-B with MELAMPODIN-C



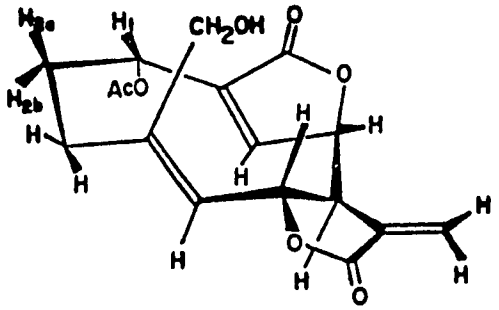
SCHEME XV

45°, an angle which would be required in a skeletal arrangement with a cis C-4(5) double bond. Thus, the second biogenetic assumption has been experimentally verified.

The assignment of the stereochemistry of the C-1 substituent was based on the torsional angles between H-1 and the H-2a and H-2b protons, with the torsional angle between H-1 and H-9 providing supporting evidence for a β-orientation of the side chain at C-1. The observed J values ($J_{1,2a}$= $J_{1,2b}$= 5.5Hz) can be explained if the C-1 hydrogen bisects the two C-2 hydrogens with approximately 45° torsional angles between H-1 and H-2a and H-2b. Dreiding models showed that there are two possible configurations, each containing two different conformations around the C_3-C_2-C_1 carbons. Contrary to published data,[29] there are two possible arrangements which could acquire 45° torsional angles. In the reported structural assignment of melampodin-B, the C-1 side chain was assigned a β-orientation where the C-2 is below the C-1, C-3 plane in the medium ring (conformation A); however, the same reported data are consistent with an α-oriented side chain at C-1 if C-2 is above the C-2, C-3



CONFORMATION A



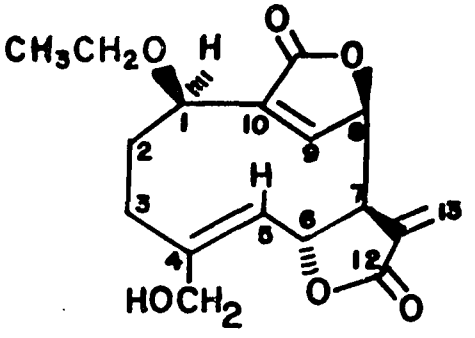
CONFORMATION B

plane of the ten-membered ring (conformation B). In order to decide which of these possibilities exist in melampodin-C, and by analogy in melampodin-B, the allylic coupling values between the C-1 and C-9 protons were analyzed. Maximum allylic coupling (approx. 3.0Hz) is observed when the two protons that are allylically coupled are perpendicular to one another.[74,76] The small allylic coupling constant ($J_{1,9}$= 1.0Hz) observed in melampodin-C indicated that the torsional angle between H-1 and H-9 should be substantially smaller than 90°. From inspection of stereomodels of the melampodin-C and melampodin-B skeleton a torsional angle of about 45° was derived which seemed to be in good agreement with the observed coupling constants. Conversely, if the medium ring contains an α-oriented C-1 substituent in conformation-B, the torsional angle between H-1 and H-9 would be about 90°; therefore, a larger value for $J_{1,9}$ should have been observed for melampodin-C and melampodin-B. On the basis of the above arguments, a β-configuration of the side chain at C-1 in 64 and 56 was assigned.

The acetate 65 and the aldehyde 66 seemed to have the same basic stereochemistry as melampodin-C since the coupling constants of the pertinent protons (i.e., H-1, H-5, H-6, H-7, H-8, and H-9) have the same or nearly the same values as the J-values of the parent compound 64. Furthermore, since the various transformations did not affect the chiral carbon centers and the gentle reaction conditions should not have changed the stereochemistry at any position in the medium ring, it was concluded that the stereochemistries of the derivatives 65 and 66 are the same as in melampodin-C.

CINERENIN

Cinerenin (67), $C_{17}H_{20}O_6$, mp 161-163°, was initially isolated from M. cinereum and later from M. argophyllum. It represented the first reported example of a germacranolide sesquiterpene lactone in which a substituent is attached to the medium ring by an ether linkage. The ir spectrum of 67 contains absorptions typical of an α,β-unsaturated γ-lactone (1775, 1750 cm^{-1}) while signals at 3450 cm^{-1} and 1665 cm^{-1} were indicative of a hydroxyl group and double bonds, respectively. The structure of cinerenin (67) was



67

inferred from the 25.2MHz ^{13}Cmr and 100MHz pmr spectral data and by correlation with melampodin-B (56). Mass spectral fragmentation patterns typical of the melampodin-B skeleton corroborated the above structural assignment while converting 67 into the previously obtained tribromide 60 gave conclusive chemical proof for the structure of cinerenin.

TABLE 6

Carbon-13 Parameters of Cinerenin (67)

Skeletal Type	Chemical Shift	Assignment
-C(C=O)-O	173.2 s	C-14
	169.6 s	C-12
$(C)_2$-C=	148.1 s	C-10
	136.6 s	C-4
	133.0 s	C-11
$(C)_2$-CH-O	79.1 d	C-8
	73.4 d	C-6
	73.2 d	C-1
C-HC=	153.9 d	C-9
	122.3 d	C-5
C-CH_2-C	28.3 tr	C-3
	23.2 tr	C-2
C-CH_2-O	65.3 tr*	C-1'
	65.1 tr*	C-15
H_2C=	122.3 tr	C-13
$(C)_2$-CH-C	49.5 d	C-7
-CH_3	15.7 q	C-2'

The spectrum was determined an a Varian XL-100-15 spectrometer operating Fourier transform mode with proton decoupling. The chemical shifts are relative to internal TMS and are recorded in ppm relative to TMS. PND, proton noise-decoupled spectrum; SFOCD, single-frequency off-center decoupled spectrum. The number of lines in the SFOCD spectrum are designated as follows: s, singlet; d, doublet; tr, triplet; q, quartet. Spectrum was obtained in acetone-d_6.

*Chemical shifts for C-1' or C-15 could not be conclusively assigned.

NMR Spectral Data of Cinerenin

The ^{13}Cmr data were obtained under the previously described conditions (page 75). Chemical shift considerations indicated that the compound contains 17 carbon atoms; therefore $C_{17}H_{20}O_6$ is the only empirical formula in agreement with both ^{13}C data and elemental analysis. Table 6 gives the assignment, chemical shift, and splitting patterns of each signal. The assignments of the chemical shifts for the respective carbon atoms of cinerenin were made by comparison with the ^{13}C data of melampodin-B, the model compound. Therefore, on the basis of chemical shift values and the SFOCD patterns of the particular signals, it was determined that cinerenin contains the following skeletal units: a) three $(C)_2$-C=, b) three $(C)_2$-CH-O, c) two -C(C=O)-O, d) two C-HC=, e) two C-CH_2-C, f) two C-CH_2-O, g) one H_2C= , h) one $(C)_2$-CH-C, i) one -CH_3 group.

The pmr spectral data of cinerenin (300MHz Table 7, and the 100MHz Figure 7) and its derivatives 68, 69, and 60 (Table 8) gave compelling evidence for the structure of cinerenin as represented in 67. The 300MHz pmr spectra of 67 were obtained in pyridine-d_5 and acetone-d_6, giving additional evidence as to the types of protons found in cinerenin. Double irradiation experiments in acetone-d_6 provided the following structural data: 1) the typical doublets at δ5.96 (H-13a) and δ6.30 (H-13b) collapsed to singlets when the multiplet at δ3.50 (H-7) was irradiated, while the triplet at δ4.53 (H-6) collapsed to a broadened doublet; 2) the broadened singlet at δ5.89 (H-8) sharpened when the multiplet at δ3.50 (H-7) was irradiated; 3) the doublet at δ5.56 (H-5)

100 MHz NMR Spectrum

of Cinerenin in Acetone - d_6

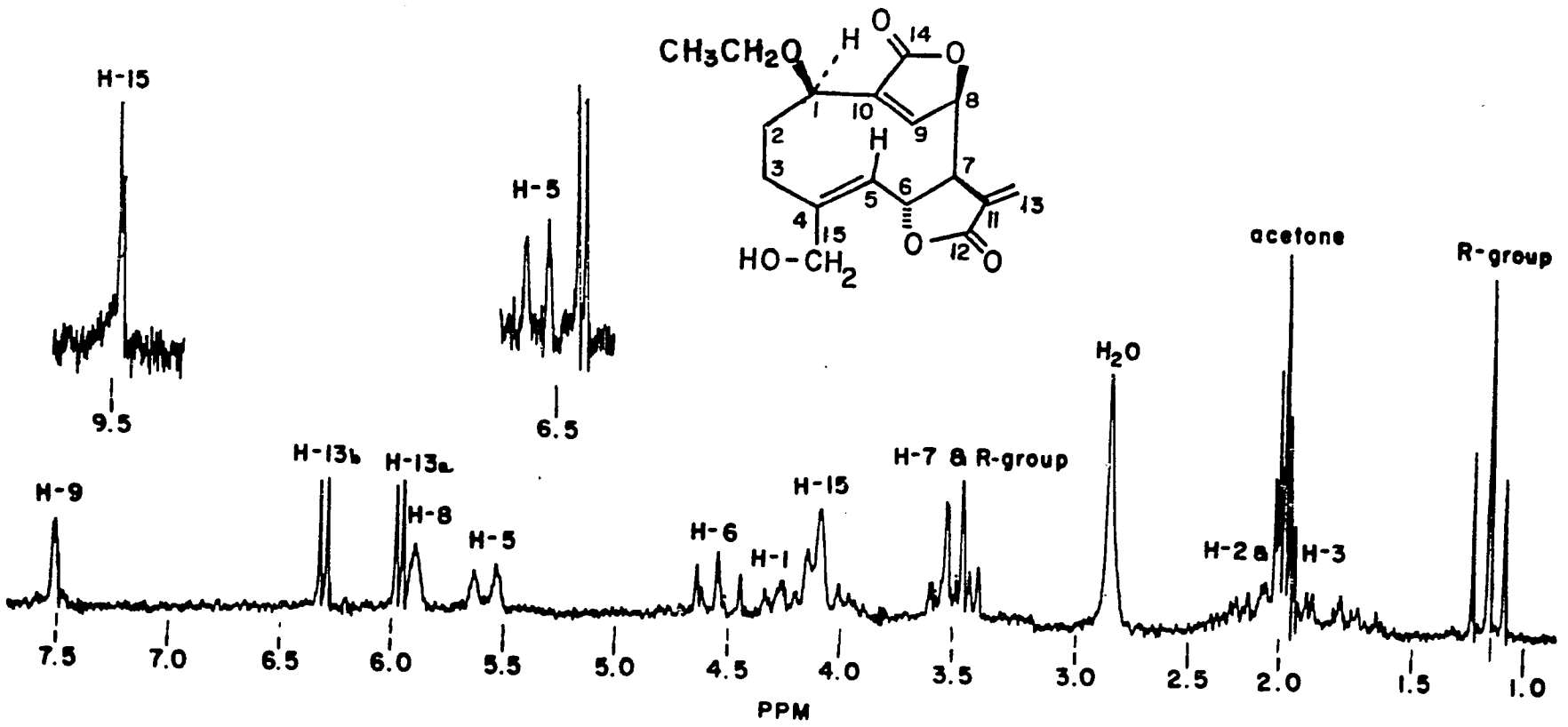


FIGURE 7

TABLE I

PMR Parameters of Cinerenin (67)

Assignment	67[b*]	67[c*]	67[a]
H-1	4.25 dd (8.0;5.8)	4.42 brdd	4.39 m
H-2a	1.71 dddd (13.5; 8.0;8.0;3.2)	1.84 m	x
H-2b	2.28 brddd (13.5; 8.0;5.8;3.2)	2.34 m	x
H-3a	1.91 ddd (15.0; 8.0;3.2)	2.08 ddd (14.5; 7.0;3.5)	x
H-3b	2.16 brddd (15.0; 8.0;3.2)	2.20 ddd	x
H-5	5.56 brd (9.5)	5.95 brd (9.0)	5.60 brd (9.5)
H-6	4.53 tr (9.5)	4.94 tr (9.0)	4.68 tr (9.5)
H-7	3.50 m	3.52 brddd (9.0;3.5;3.0)	3.20 m
H-8	5.89 brs	5.97 brs	5.61 brs
H-9	7.52 brs	7.58	7.25 d (1.0)
H-13a	5.96 d (3.0)	5.96 d (3.0)	5.84 d (3.0)
H-13b	6.30 d (3.5)	6.51 d (3.5)	6.48 d (3.5)
H-15	4.09 brs (AB pattern)	a) 4.30 brd (15.0) b) 4.39 brd (15.0)	4.15 brs
C-2' Me	1.14 tr (7.0)	1.11 tr (5.5)	1.23 tr (7.0)
C-1' CH_2-	3.49 m (7.0)	3.42 dq (7.0; 2.0)	3.57 dq (7.0; 2.0)

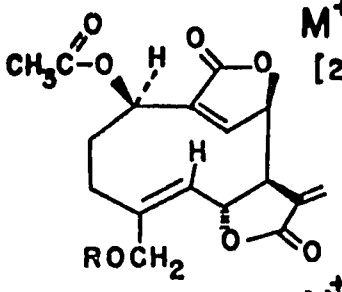
a = $CDCl_3$; b = acetone-d_6; c = pyridine-d_5; * 300MHz Spectra.

collapsed to a broadened singlet and the H-7 multiplet sharpened when the H-6 triplet at δ4.53 was irradiated; 4) when the signal at δ7.52 (H-9) was irradiated the signal at δ4.25 (H-1) and the broadened singlet at δ5.89 (H-8) both sharpened; 5) the triplet at δ1.14 (C-2') collapsed to a singlet when the multiplet at δ3.49 (C-1') was irradiated and irradiation of the C-2' triplet collapsed the C-1' multiplet. Cinerenin was shown to be a primary allylic alcohol by observing a downfield shift of the two proton absorption at δ4.09 (H-15) in 67 to δ5.03 in cinerenin acetate (68). In addition, allylic coupling was observed between H-5 and the H-15 signals in 67, 68, and 69.

Mass Spectral Data of Cinerenin and Derivatives

The mass spectral fragmentation patterns of cinerenin (67) and the acetate (68) show peaks at m/e 274, 256, and 228, typical of the melampodin-B type dilactone skeleton, in addition to their respective parent peaks at m/e 320 and 362 (Scheme XVI). The ethoxy group at C-1 in 67 is lost as ethanol (M^+ - 46) resulting in a major fragment at m/e 274, a fragment which is typical for the melampodin-B skeleton. Further loss of H_2O (18 m.u.) leads to the m/e 256 fragment while additional loss of CO (28 m.u.) gives the fragment m/e 228. In cinerenin acetate (68) the peak at m/e 319 (M^+ - 43) is due to the loss of an acylium ion (CH_3CO^+) from the parent compound while the peak at m/e 316 (M^+ - 46) results

MASS SPECTRAL DATA of MELAMPODIN B-TYPE COMPOUNDS (C)



R = H
R = Ac

M^+ 376 [21.8] $-43\,(CH_3C{\equiv}\overset{+}{O})$ → 333 [26.5]

-59 ↙ (CH_3COO)

274 [97.0] [53.5] $-18\,(H_2O)$ → 256 [100] [100]

-28 ↓ (CO)

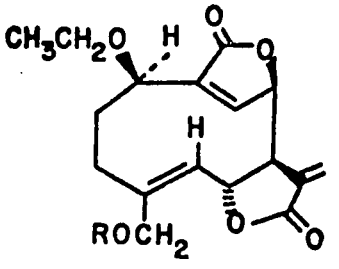
228 [93] [96.5]

M^+ 334 $-60\,(CH_3COOH)$

M^+ 362 [5.3] $-43\,(CH_3C{=}\overset{+}{O})$ → 319 [5.2]

-45 ↓ (C_2H_5O)

-46 ↓ $(HOCH_2CH_3)$



R = H
R = Ac

316 [29.8] -42 → 274 [16.2] [71.1] $-18\,(H_2O)$ → 256 [100] [100]

-28 ↓ (CO)

228 [114] [82.2]

M^+ 320 [4.45] $-46\,(CH_3CH_2OH)$

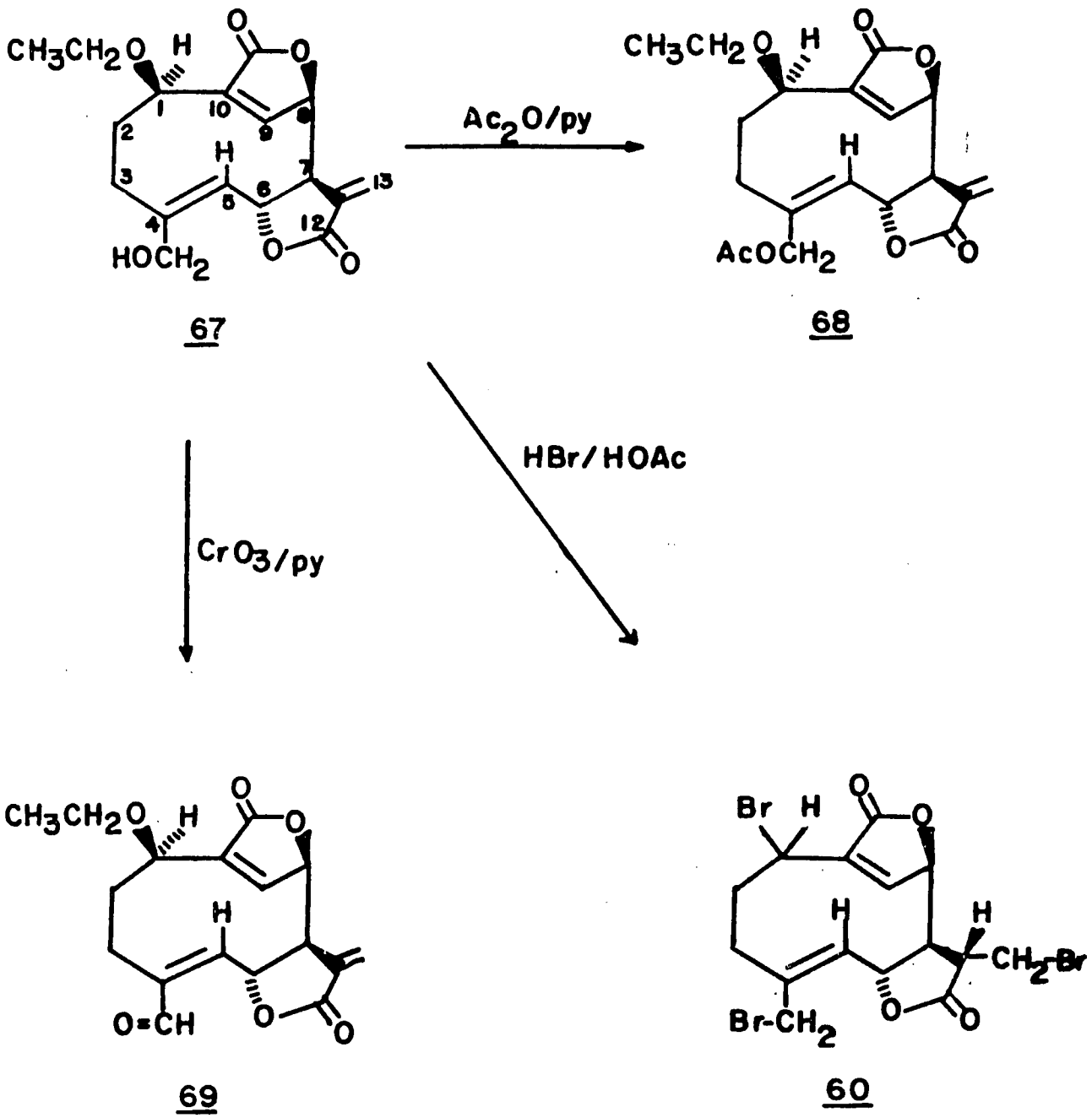
SCHEME XVI

from the loss of ethanol. The intense peak at m/e 274 (M^+ - 88) in 68 could be caused by the sequential or simultaneous loss of ethanol (46 m.u.) and CH_2=C=O (42 m.u.). The further loss of H_2O results in the peak at m/e 256 while the additional loss of CO leads to the m/e 228 fragment.

Cinerenin Derivatives

Cinerenin was transformed into 68, 69, and 60 (Scheme XVII), the elemental analyses of which confirmed $C_{17}H_{20}O_6$ as the empirical formula of 67. Derivatives 68 and 69 gave evidence for the presence of a primary allylic alcohol at C-15 in cinerenin for the following reasons: a) the H-15 signal at δ4.30 and δ4.39 in 67 appeared downfield as a doublet at δ5.03 in 68; b) one acetyl methyl signal at δ1.98 was observed in 68: c) the H-5 doublet at δ5.95 in 67 was found at δ6.45 in 69; d) the H-15 proton absorptions in 67 were lost upon oxidation with Sarett's reagent and a signal at δ9.40, typical of an aldehydic proton, appeared in 69; e) allyic coupling between H-5 and H-15 was observed in 67, 68, and 69. Cinerenin was also converted to the tribromide 60. Although 67 does not have an ester function at C-1, as do 56 and 64, the reaction conditions (HBr/HOAc) also favor cleavage of ether bonds. By analogy with Zeisel's method, the reaction proceeds by protonation of the ether oxygen followed by a nucleophilic attack of bromide at C-1 either by a S_N1 or S_N2 process. This chemical conversion, together with the nmr data, gave compelling evidence for the identical skeletal

CINERENIN DERIVATIVES



67 68 69 60

SCHEME XVII

TABLE 8

PMR Parameters of Cinerenin Derivatives

Assignment	68[a]	68[c]	69[b]	69[c]
H-1	4.33 m	4.35 m	4.23 m	4.34 m
H-5	5.47 brd (9.5)	5.62 d (9.5)	6.58 d (9.5)	6.45 d (9.5)
H-6	4.60 tr (9.5)	5.32 tr (9.5)	4.72 tr (9.5)	5.00 tr (9.5)
H-7	3.16 m	3.50 m	3.78 m	3.77 m
H-8	5.59 brs	5.41 brs	5.97 brs	5.95 s
H-9	7.24 d (1.0)	7.50 d (1.0)	7.62 d (1.0)	7.60 d (1.0)
H-13a	5.82 d (3.0)	5.90 d (3.0)	6.05 d (3.0)	5.95 d (3.0)
H-13b	6.48 d (3.5)	6.41 d (3.5)	6.38 d (3.5)	6.45 d (3.5)
H-15	4.60 d (1.5)	5.03 brd (1.5)	9.46 d (1.5)	9.40 d (1.5)
C-1'-CH_2-	3.53 dq (7.0;3.0)	3.43 dq (7.0;2.0)	3.44 dq (7.0;3.0)	3.40 pt (7.0;2.0)
C-2' Me	1.21 tr (7.0)	1.09 tr (7.0)	1.07 tr (7.0)	1.05 tr (7.0)
Acetyl	2.12 s	1.98 s		

a = $CDCl_3$; b = acetone-d_6; c = pyridine-d_5; All spectra are 100MHz.

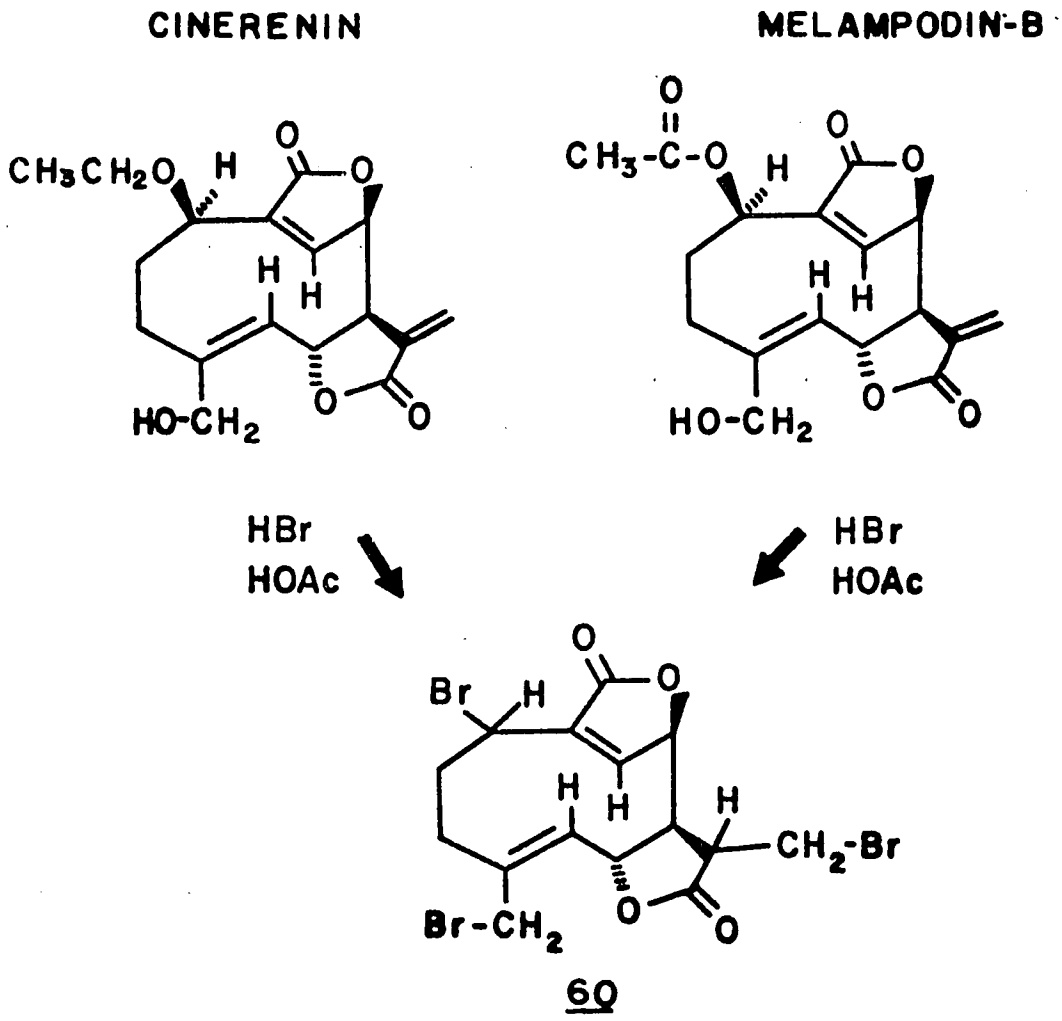
structures in the medium ring portion of cinerenin (67), melampodin-B (56), and melampodin-C (64) (Scheme XVIII).

Stereochemistry of Cinerenin and Derivatives

Unlike melampodin-B and melampodin-C, cinerenin contains a C-1 ether substituent, while ester functions at C-1 are present in 56 and 64. As a result, the stereochemistry at C-1 in 67 may be changed in the biosynthesis of 67. In the ir spectra of 56 and 64, the OH absorptions at about 3450 cm^{-1} appear as sharp peaks while in cinerenin the OH absorption at 3450 cm^{-1} is broadened. It can be argued that the alcohol group at C-15 undergoes intramolecular hydrogen bonding involving both the C-14 and more favorably the C-1' carbonyl functions in 56 and 64, while in cinerenin only the C-14 carbonyl group is available; thus, the OH absorption in 67 is broadened due to a less rigidly held conformation causing a weakening of the hydrogen bonding between the carbonyl group at C-14 and the hydroxyl group at C-15. The broadening of the OH peak in 67 may also be caused by intermolecular hydrogen bonding which, due to a stronger intramolecular association does not occur in compounds 56 and 64. Therefore, because the C-15 hydroxyl substituents have a β-orientation in 56 and 64, the C-1 substituents in these compounds must also be β-oriented in order for the postulated intramolecular hydrogen bonding to occur.

The small allylic coupling between the H-1 and H-9 signals in cinerenin ($J_{1,9}$= 1.0Hz) indicated that the torsional angle

CORRELATION of MELAMPODIN-B with CINERENIN



60

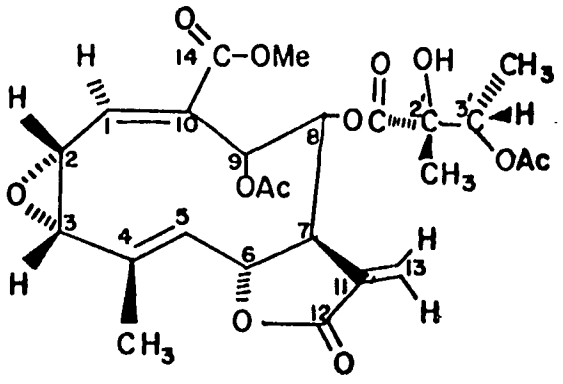
SCHEME XVIII

between the H-1 and H-9 is less than 90°, as found in the structures of melampodin-B and melampodin-C. This condition is met only when the substituent at C-1 in 67 is β-oriented; that is, the ethoxy group at C-1 in cinerenin must have a β-orientation. Since both melampodin-B and cinerenin have the same configuration at C-1, it is less likely that the ethoxy group is biosynthetically introduced by a substitution reaction rather than by a reductive process at the acetoxy carbonyl carbon. Enzymatic reduction of carbonyl groups are rare in the biosynthesis of terpenoid derivatives and to the best of my knowledge, cinerenin represents the first sesquiterpene lactone which has an ether-linked side chain.

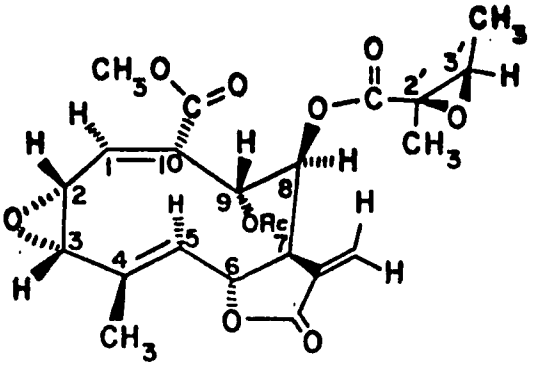
The stereochemistry of the medium ring skeleton of cinerenin can be considered identical to that of 56 and 64 since the coupling constants of the H-1, H-5, H-6, H-7, H-8, and H-9 in these respective compounds are almost the same for the corresponding proton interactions. This was particularly evident in the similarity of the 300MHz pmr spectral patterns of the crucial protons signals H-1, H-2a, and two H-3 protons (compare Table 2 and Table 7). Extending the preceding considerations to the derivatives of cinerenin (i.e., the acetate 68 and the aldehyde 69) substantiated the same molecular stereochemistry for each of these compounds.

MELAMPODININ

Previously, sesquiterpene lactones could not be detected in a number of species of the yellow-rayed complex of Melampodium.* Therefore, it was of particular interest to investigate the yellow-rayed species M. americanum which is morphologically and cytologically most closely related to the white-rayed complex. Although the terpenoid-containing extracts were obtained in lesser amounts (as compared to the white-rayed species), a new sesquiterpene lactone was isolated from M. americanum which we named melampodinin (70), $C_{25}H_{30}O_{12}$, mp 208-210°. This first reported sesquiterpene lactone isolated from a yellow-rayed species of Melampodium shows ir absorptions (nujol) at 3450 cm^{-1} and 1770 cm^{-1} which are typical of hydroxyl groups and γ-lactones, respectively. The structure of melampodinin (70) was determined by comparing its 100MHz pmr spectral



70



71

*N. H. Fischer, unpublished data.

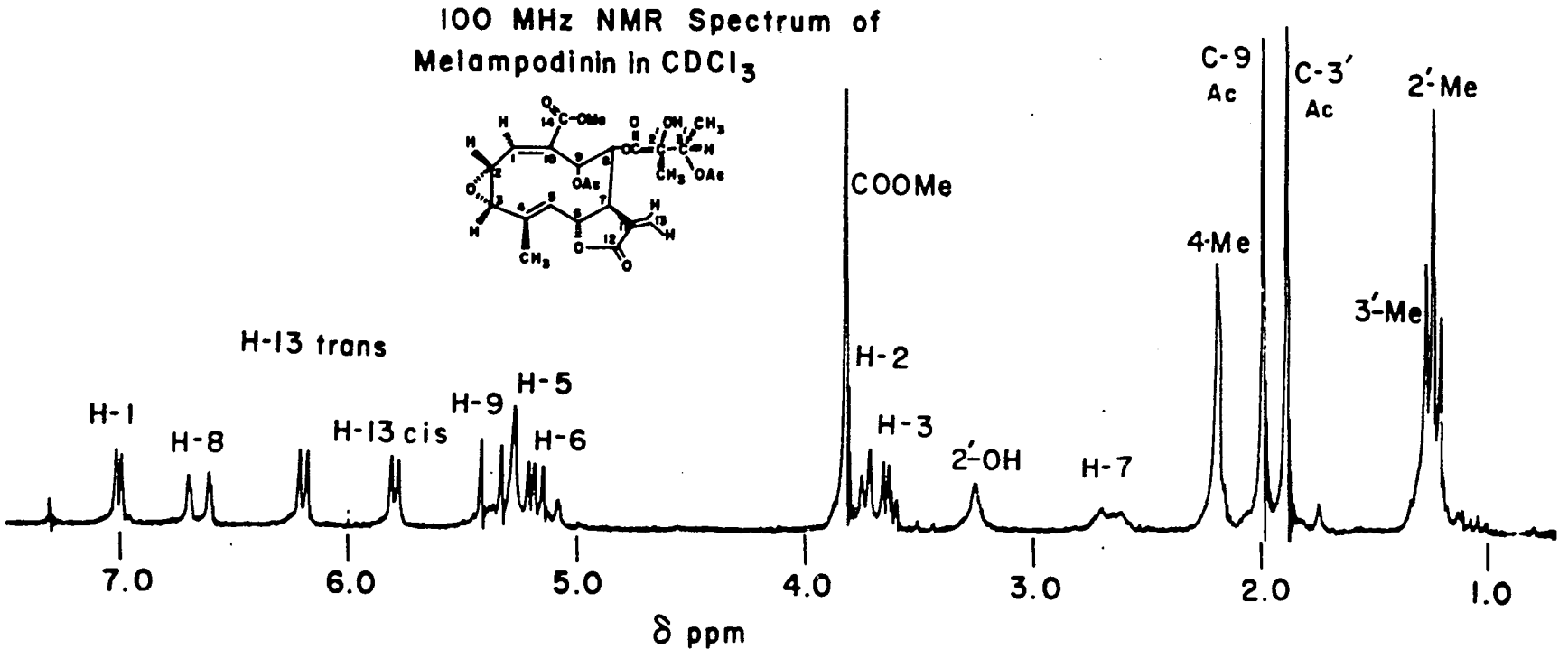

100 MHz NMR Spectrum of
Melampodinin in CDCl3
H-1
H-8
H-13 trans
H-13 cis
H-9
H-5
H-6
COOMe
H-2
H-3
2'-OH
H-7
4-Me
C-9
Ac
C-3'
Ac
2'-Me
3'-Me
7.0
6.0
5.0
4.0
3.0
2.0
1.0
δ ppm

FIGURE 8

TABLE 9

PMR Parameters of Melampodinin (70) and Melampodin-A Acetate (71)

Assignment	70[a]	70[c]	71[a]
H-1	7.00 dd(1.0; 2.3)	7.18 x	7.04 dd(1.0;2.5)
H-2	3.62 dd(2.3; 3.6)	3.88*	3.65 dd(2.5;3.8)
H-3	3.73 dd(1.0; 3.6)	3.83*	3.75 dd(1.0; 3.8)
H-5 & 6	5.07-5.38*	5.56 dd(9.0;9.0)	5.10-5.42*
H-7	2.65 m	3.23 m	2.73 m
H-8	6.65 dd(1.2;9.0)	7.15 dd(1.2;9.0)	6.70 dd(1.2;9.0)
H-9	5.37 d (9.0)	5.66 d (9.0)	5.41 d (9.0)
H-13a	5.79 d (3.0)	5.95 d (3.0)	5.75 d (3.0)
H-13b	6.19 d (3.5)	6.26 d (3.5)	6.25 d (3.5)
H-3'	5.18 q (6.0)	5.56 q (6.5)	3.05 q (5.5)
C-4 Me	2.19 d (1.0)	2.27 brs	2.18 d (1.0)
C-2' Me	1.25 s	1.49 s	1.48 s
C-3' Me	1.24 d (6.0)	1.40 d (6.5)	1.20 d (5.5)
C-9 Ac	1.99 s	1.87 s	2.00 s
CO_2Me	3.82 s	3.67 s	3.85 s
C-3' Ac	1.89 s	1.78 s	
C-2' OH	3.24 s	4.78 s	

---

a = $CDCl_3$; c = pyridine-$\underline{d}_5$; x = obscured by solvent signal

*Overlapping part of ABX system; Spectra are 100MHz.

parameters with those of melampodin-A acetate (71)[71] which are nearly identical (Figure 8, Table 9).

PMR Spectral Parameters of Melampodinin

The gross similarities between the 100MHz pmr spectra of 70 (Figure 8) and melampodin-A acetate 71[71] immediately indicated a close resemblence in their molecular structure. In the spectrum of 70, two additional peaks are present which were not observed in melampodin-A acetate (71), a three proton singlet at δ1.89 and a one proton singlet at δ3.24. Since all the skeletal proton signals in 70 can be assigned by comparison with 71, it was inferred that the additional singlets are due to the substituent at C-8. Since the C-8 side chain in 71 contains a C-2', C-3' epoxide group, it can be envisioned that the following steps could have taken place in the biosynthesis of 70 from 71: the addition of an acetate group at C-3', with subsequent epoxide ring opening, formed an alcohol at C-2'. The presence of an acetoxy alcohol at the side chain of 70 was indicated by a three proton singlet at δ1.89 (acetate) and a one proton singlet at δ3.24 (OH). The reaction can be envisioned as a stereospecific nucleophilic addition of an acetate to the β-position of an α,β-epoxy ester. If this were the case, the stereochemistry of 70 should be as indicated in Figure 70, since the acetate attack at the C-3' epoxide carbon would most likely proceed with inversion of configuration at C-3' and retention of configuration at C-2'.

The following spin-spin decoupling experiments verified the structure of melampodinin (70): 1) irradiation of the multiplet at

δ2.65 (H-7) collapsed the doublets at δ5.79 (H-13a) and δ6.19 (H-13b) to singlets while simplifying the multiplet at δ5.07-5.38 (H-6); 2) when the signal at δ7.00 (H-1) was irradiated the signal at δ3.62 (H-2) collapsed to a doublet; 3) spin-decoupling of the signal at δ6.65 (H-8) collapsed the doublet at δ5.39 (H-9) to a singlet and simplified the H-7 multiplet at δ2.65; 4) irradiation of the methyl doublet at δ1.24 (C-3') collapsed the signal at δ5.18 (H-3') while irradiation of the H-3' signal collapsed the C-3' methyl doublet to a singlet.

The identities of the carbomethoxy group at C-10, the acetoxy groups at C-9 and C-3', and the C-2' hydroxyl group were verified by obtaining pmr spectra in pyridine-$\underline{d}_5$ using $CDCl_3$ as a reference solvent. The different solvents produce characteristic differences between the chemical shift values of the respective groups. It was found that in pyridine-$\underline{d}_5$ the C-9 acetoxy signal was shifted upfield by 0.12 ppm while the C-3' acetoxy signal shifted upfield by 0.11 ppm. Similarly, the signal due to the carbomethoxy group at C-10 shifted 0.15 ppm upfield. These characteristic shifts are due to the different electronic environments around these substituents as a result of the orientation and complexation of the pyridine molecules to the carbonyl functions of these groups.[75] The large downfield shift by 1.54 ppm of the C-2' OH proton is due to the hydrogen bonding between the OH and pyridine-$\underline{d}_5$ molecules.

Consequently, the pmr experiments together with the elemental analysis substantiated the structure of melampodinin with the tentative stereochemical assignment at C-2' and C-3' as shown in 70.

An Incomplete Structure Determination of a New Sesquiterpene Lactone from M. cinereum

In the populational collection #2013 (Stuessy/Fischer) of M. cinereum, a new germacranolide sesquiterpene lactone was isolated which in the following discussion will be designated as "2013". Due to the limited amount of this compound, critical experiments could not be completed; therefore the structure elucidation of "2013" is only partially resolved.

Compound "2013", a colorless crystalline material, mp. 194-195°, shows ir absorptions (nujol) at 3500 cm^{-1} (OH), 1765 cm^{-1} (γ-lactone), 1720 (α,β-unsaturated ester), and 1660 cm^{-1} (double bond). These ir data are similar to the ir spectra of the other compounds previously described, and it was assumed that this compound contains an alcohol function as well as a γ-lactone group. It was confirmed that "2013" contains one primary alcohol function since a pmr signal typical of a methylene group in "2013" appears more downfield in the acetate of "2013"; furthermore, the ir spectrum of the acetate of "2013" lacks an OH absorption.

The 100MHz spectra of "2013" (Table 10) contain the doublets typical of an α,β-unsaturated γ-lactone at δ5.73 and δ6.14. Furthermore, the three-proton singlet at δ3.76 suggested that a carbomethoxy substituent is present in the molecule. This assignment was verified by the following observations: 1) melampodin-A and several analogs have carbomethoxy substituents which absorb at about

TABLE 10

PMR Parameters of "2013" and "2013" Acetate

Assignment	"2013"[b]	"2013" acetate[e]	"2013" acetate[a]
H-1	6.87 dd (5.0; 12.0)	6.65 dd (5.0; 12.0)	6.88 dd (5.0; 12.0)
H-7	3.02 m	2.90 m	3.00 m
H-8 or H-6	5.51 m	5.61 m	5.50 m
H-8 or H-6	5.23 m	4.74 m	5.10 m
H-13a	5.73 d (1.5)	5.08 d (1.5)	5.72 d (1.5)
H-13b	6.14 d (2.0)	6.11 d (2.0)	6.32 d (2.0)
H-15	3.36 m	3.54 m	3.89 d (5.0)
CO_2Me	3.76 s	3.48 s	3.83 s
C-1' Me	a) 1.07 d (7.0)	a) 1.07 d (7.0)	a) 1.13 d (7.0)
	b) 1.05 d (7.0)	b) 1.06 d (7.0)	b) 1.11 d (7.0)
H-1'	2.44 m	2.40 m	2.50 m
Acetyl		1.66 s	2.06 s 2.10 s

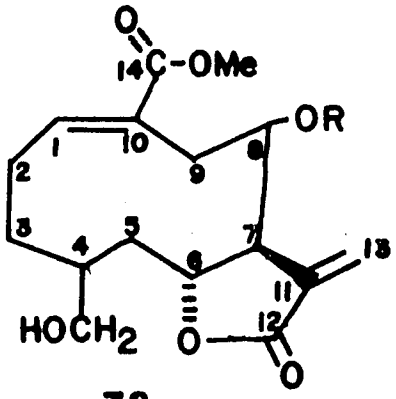
a = $CDCl_3$; b = acetone-d_6; e = benzene-d_6; Spectra are 100MHz.

δ3.80, and a similar absorption was found in "2013"; 2) when the pmr spectrum was obtained in benzene-$\underline{d}_6$ a characteristic upfield shift of the carbomethoxy group by an increment of about 0.35 ppm was observed, a result that was also found for melampodin-A.

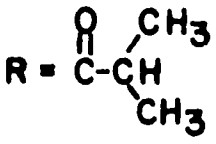
The doublet of doublets at δ6.87 in "2013" is typical of the β-hydrogen in an α,β-unsaturated ester and similar to the H-1 signal observed in melampodin-A. On this basis, this signal in "2013" was assigned H-1. The pair of three proton doublets at δ1.07 and δ1.05 suggested that an isopropyl group is present in "2013", analogous to melampodin-C. Subsequent decoupling experiments verified this assigmment, as well as the assigmments for the H-1, the two H-13, H-7, and H-1 signals.

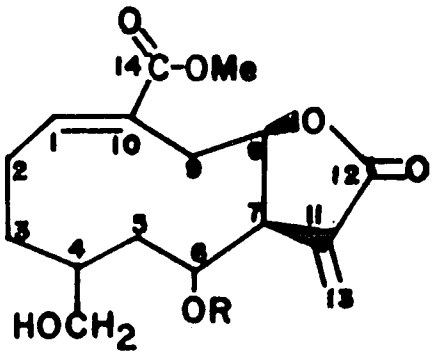
At this point, it was apparent that "2013" is structurally more closely related to melampodin-A than to melampodin-B for the following reasons: 1) the observed region of the H-1 and carbomethoxy signals in "2013" are similar to those of melampodin-A, and 2) the mass spectral fragmentation patterns of "2013" do not include the fragments m/e 274, 256, and 228 which are typical of melampodin-B type compounds. Elemental analysis indicated an empirical formula of $C_{20}H_{28}O_7$ and mass spectra gave supporting evidence (parent peak at m/e 380 in "2013" and m/e 422 in the acetate of "2013"). It must be noted that the confirmation of this empirical formula was not unequivocal since the acetate of "2013" did not crystallize and, therefore, reliable elemental analysis of this compound were not obtained; also the mass spectra were not high resolution mass spectra.

Structures 72 and 73 are consistent with the limited experimental data of "2013" and its acetate. Decoupling experiments



72





73

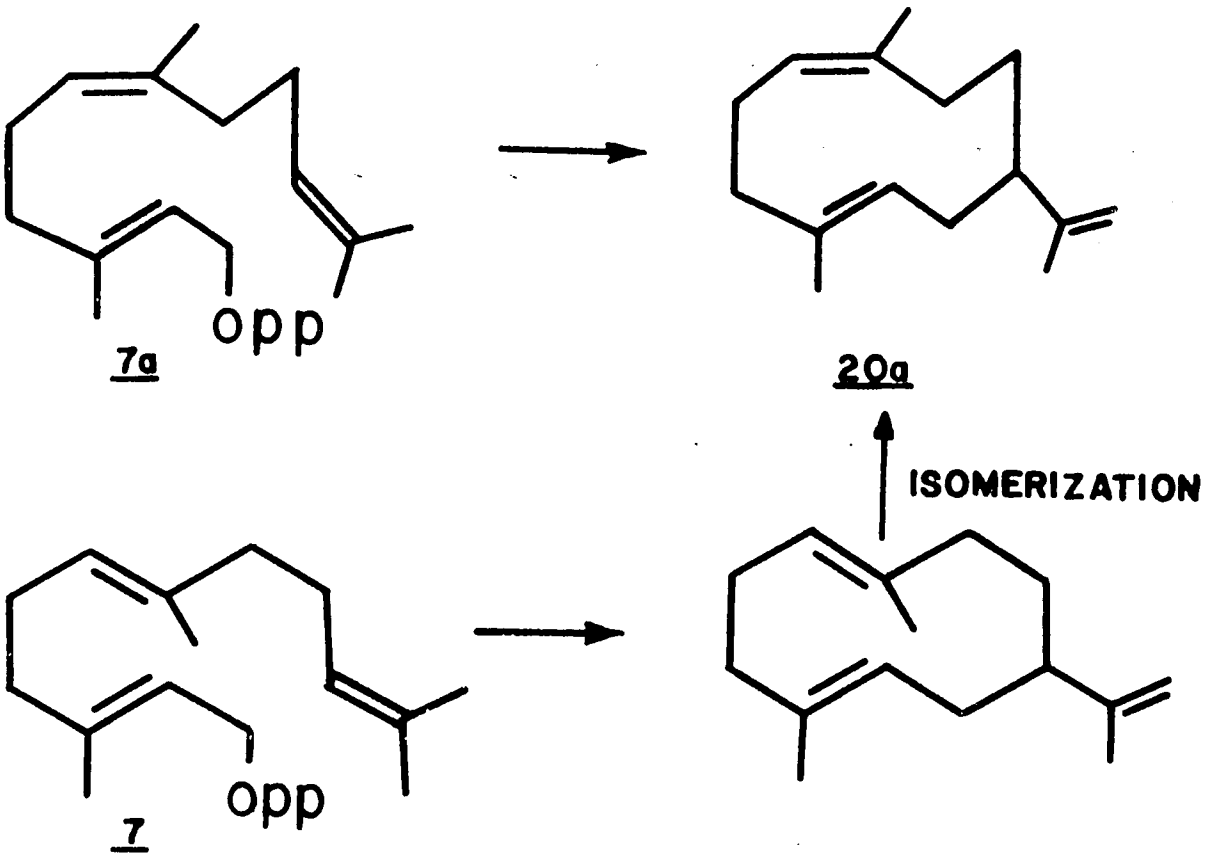
on "2013" and the acetate of "2013" presented insufficient evidence to allow a choice to be made between 72 and 73 because the signals for H-8 and/or H-6 at δ5.51 and δ5.23 are coupled to the two proton signals of H-5 and/or H-9. These latter signals were observed in the same region of the 100MHz pmr spectra (δ1.25-2.00); due to the overlapping of the signals for H-5 and H-9, conclusive assignments were impossible. Decoupling experiments on a 300MHz pmr spectrum may prove more useful since many signals in the 100MHz spectra are obscure due to overlap.

In contrast to other melampodin-A type compounds, the coupling constants of the C-13 methylene protons in "2013" are lower ($J_{13a,7}$= 1.5Hz and $J_{13b,7}$= 2.0Hz); consequently, structure 73 seemed to be more likely for "2013" since it is well documented in the literature[24] that the coupling constants for C-13 methylene protons are usually smaller in 7,8-lactones than in 7,6-lactones. By comparison, the coupling constants for H-13a, H-7, and H-13b, H-7 in

7,6-lactones such as melampodin-A are 3.0Hz and 3.5Hz, respectively. Furthermore, examples cited in a recent collection of sesquiterpene lactone pmr spectra[24] seem to corraborate this assumption, although no conclusive evidence is intended to be given by these observations.

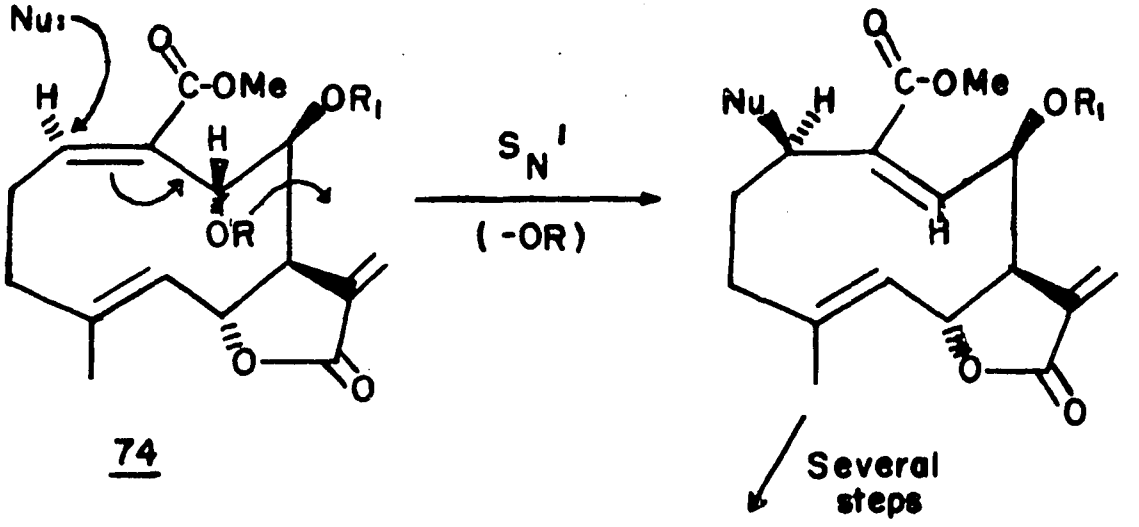
Possible Biosynthesis and Interconversion of Melampodin-A and Melampodin-B Type Compounds

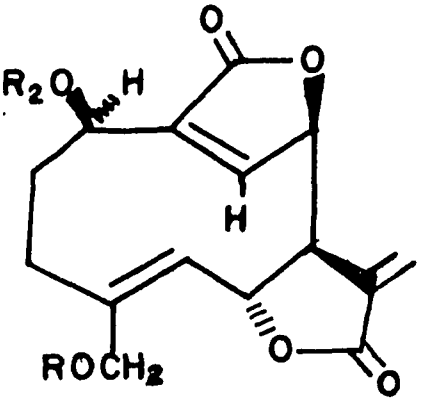
The germacranolides are believed to be biosynthesized by the cyclization of trans,trans farnesyl pyrophosphate (7) (Introduction, Scheme V). Neither melampodin-A (17) nor melampodin-B (56), is a trans,trans-cyclodeca-1,5-diene germacranolide, but are biomodified versions of the cyclodecadiene medium ring. Thus the biosynthesis of the melampolides (Introduction, page 13) can be hypothesized to involve the following possible routes:



SCHEME XIX

In one pathway, trans,trans farnesyl pyrophosphate (7) is cyclized to the trans,trans-germacrolide skeleton, followed by trans,cis-isomerization of the C-1(10) double bond resulting in the melampolide ring skeleton (20a). In the alternative route, cis, trans farnesyl pyrophosphate (7a) is converted directly to the melampolide ring (20a). Bio-conversions of melampodin-A type compounds (74) to the dilactone melampodin-B and its analogs can be formulated as S_N' reactions followed by other bio-modification processes. (Scheme XX).





75

SCHEME XX

The stereochemical consequences of this transformation can be rationalized on the basis of the following arguments: Melampodin-A was found to adopt a conformation in which the carbomethoxy group at C-10 and the C-1 hydrogen are α-oriented in relation to the plane of the medium ring.[29,31] If melampolides in general adopt a conformation in which the substituents around C-1 and C-10 are below the plane of the medium ring, then for steric reasons attack of any reagent could only occur from the outer face of the C-1(10)-cis double bond. Inspection of stereomodels of melampodin-A showed that the C-9 hydrogen and the carbomethoxy group at C-10 have an anti-periplanar orientation. In a S_N' type process, a nucleophilic attack at C-1 of the melampolide ring (74) with subsequent migration of the double bond would automatically lead to a C-9(10)-trans double bond and a β-oriented substituent (Nu:) at C-1, configurations that are typical of the melampodin-B skeleton. Lactonization of the C-14 carboxyl group toward C-8 and bio-modifications at C-15 provides the typical melampodin-B system. (It should be re-emphasized at this point that the pathways suggested above lack experimental evidence). However, the aforementioned transformation of 74 into 75 corroborates the assigned C-9 trans double bond and the β-orientation of the substituents at C-1 in melampodin-B, melampodin-C, cinerenin, and their respective derivatives. Another important implication of this bio-transformation is that plants producing melampodin-B type compounds are at a higher evolutionary level than are those providing melampodin-A type sesquiterpene lactones. It can be argued that additional enzymes are needed for a bio-conversion

of 74 into 75. If this assumption is accepted, then the chemical data (i.e. the number and type of sesquiterpene lactones) obtained from the various plant extracts can be helpful in establishing evolutionary relationships in a particular plant complex. These implications will be discussed in the following section concerning the biochemical systematics of the genus Melampodium.

PART II

The Biochemical Systematics

of

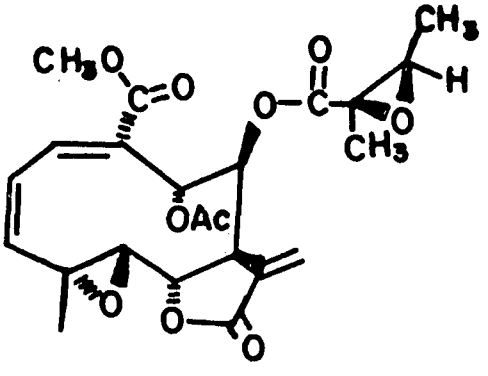
M. cinereum and *M. argophyllum*

Over the past two decades there has been a rapidly increasing surge in interest and work in the area of biochemical systematics. It was realized by scientists that chemical data may provide additional independent information in making taxonomical decisions. In addition to traditional morphological and cytological taxonomy, biochemical systematics can be helpful in solving evolutionary problems; that is, natural systematic relationships at the populational, species, and generic level. Sesquiterpene lactones have previously been successfully used in biochemical systematics of Compositae.[24,62,66]

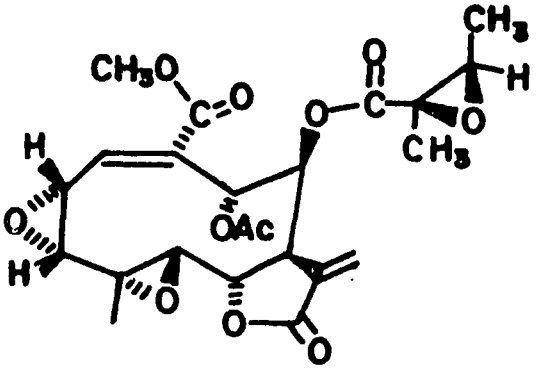
A biochemical systematic study of the genus Melampodium in the family Compositae (Heliantheae) was initiated by Stuessy.[76] Of the 37 species of Melampodium, only three species, which are confined to the southwestern United States and adjacent northern Mexico, have white-rayed flowers; the other 34 species have yellow-orange flower rays.[67] The white-rayed complex of Melampodium represented a taxonomically controversial group; various authors treated this complex as containing one, two, or three species.[68] Stuessy used cytological and morphological data together with two-dimensional paper chromatograms of leaf phenolics (not yet chemically identified) to support the assignment of three species in the white-rayed complex: M. leucanthum Torr. & Gray, M. cinereum DC., and M. argophyllum (Gray & Robinson) S. F. Blake. On the basis of this data Stuessy suggested the following possible evolutionary relationships in the white-rayed complex: from an ancestral stock in the mountains of northern Mexico, M. cinereum may have diverged and

populated south-central Texas below the Edward's plateau. Another evolutionary branch may have diverged and spread northwestward toward west Texas, New Mexico, Arizona, and Colorado to become M. leucanthum. Stuessy considered M. argophyllum to be more closely related to M. leucanthum than to M. cinereum. We felt that a populational analysis of the three-white-rayed species using sesquiterpene lactones as biochemical systematic markers could help clarify the systematic relationships in the white-rayed complex.

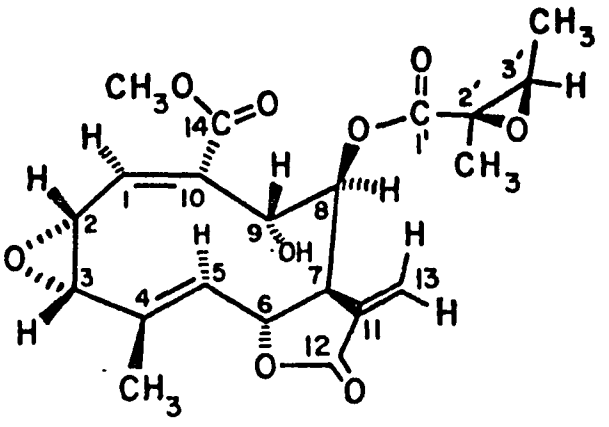
A detailed chemical analysis of the sesquiterpene lactones from M. leucanthum was initiated in our laboratory and is still in progress. A populational investigation of over 100 different populations of M. leucanthum by Carolyn Fishback and Russel Wiley has shown that melampodin-A (17) and the biogenetically closely related leucanthin-A (76) and leucanthin-B (77) are typical of M. leucanthum.[41]



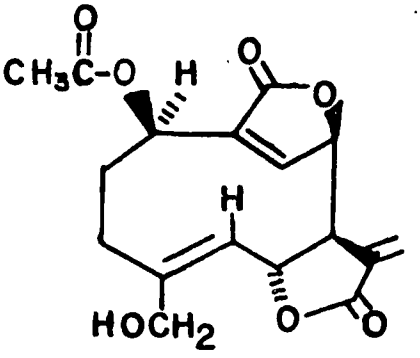
76



77



17



56

In contrast, sesquiterpene lactones of the melampodin-B type seem to be typical M. cinereum and M. argophyllum.

The following discussion describes the methods and results of the populational analysis of about 50 populations of the two species (Figure 9).

The dried leaves of each population were weighed and extracted according to Scheme IX. The resulting crude syrup was allowed to stand for at least two weeks. If crystals were formed, the syrup was filtered. In most cases melampodin-B crystallized from the crude syrup. Consequently, reliable values could be reported for the amount of this compound in each population. Next, pmr spectra were obtained of both the crystal and the crude syrup. Finally, the crude syrup was either chromatographed on a silica gel column (10 populations), or analyzed by tlc (39 populations) using the following solvent systems: propyl acetate; 9:1 (CH_2Cl_2/EtOAc); 1:9 (CH_2Cl_2/EtOAc); and 1:1 (CH_2Cl_2/EtOAc).

The populations which were analyzed by column chromatography (Table 11) provided quite reliable data. In contrast, the tlc

Populational Distribution of *M. cinereum* and *M. argophyllum*

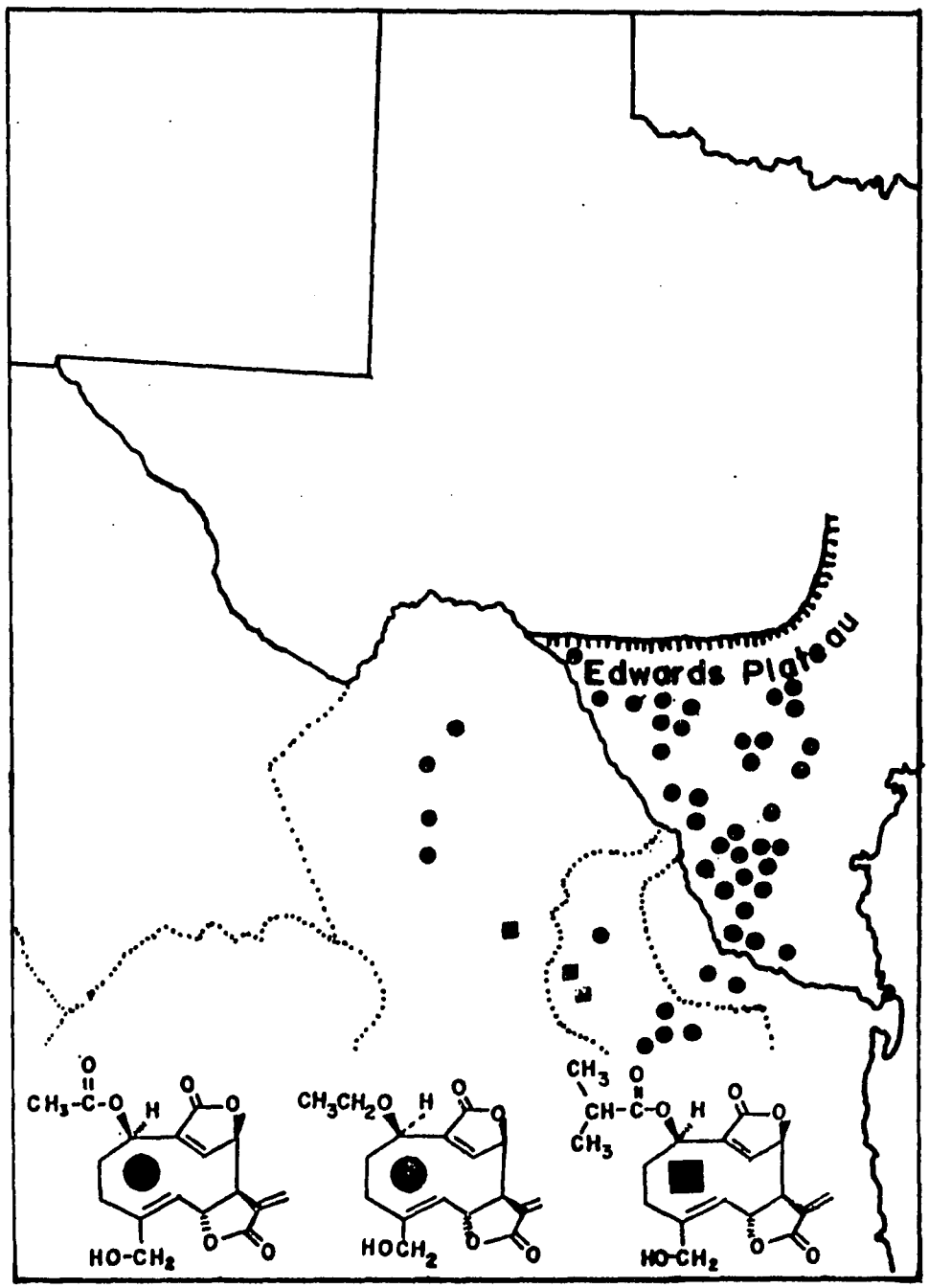
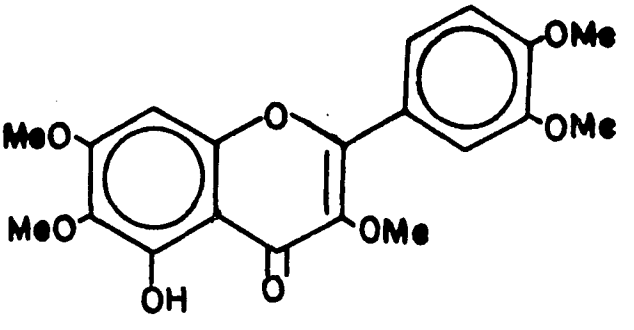


FIGURE 9

and pmr analyses of the remaining populations (Table 12 and Table 13) are less reliable due to a number of complicating factors. The most serious problems resulted from the overlap of characteristic pmr signals in the poorly resolved spectra of the crude syrups containing the respective sesquiterpene lactones as well as the proximity of the rf values in tlc runs of these extracts. In addition, streaking was observed in the tlc analyses. For each of the following compounds which were found in the populational analysis of M. cinereum and M. argophyllum the data and methods employed in its detection are described:

1. Artemetin (78), a yellow crystalline flavone, has the highest rf value of any of the compounds isolated from the crude syrups. Consequently, its detection was reliable.



78

2. 4(5)-Dihydromelampodin-B (61) was primarily detected by the presence of the H-9 signal at δ8.03 in the pmr spectra of the crude syrups and/or crystals isolated from the particular crude syrups. This procedure could in principle be employed since the H-9 signal

of 61 appears most downfield in the pmr spectra of any of the sesquiterpene lactones found in these populations. However, due to low concentrations and poorly resolved pmr spectra of the crude syrups, the detection of 61 was particularly difficult. Furthermore, the rf value for 61 is slightly greater than that of melampodin-B. Therefore, the use of tlc data for the detection of 61 proved also unreliable.

3. Cinerenin (67) shows an rf value between those of melampodin-B and artemetin. This characteristic allowed for fairly reliable detection although streaking in the tlc was frequently observed. In the pmr spectra, the characteristic signals of 67 overlap with those of melampodin-B and melampodin-C making this method doubious, at best, for distinguishing between these three sesquiterpene lactones.

4. Melampodin-C (64), a white crystalline germacranolide, was the hardest to detect unambiguously since its rf value is almost the same as that of compound "2013". The characteristic pmr signals of 64 overlap with those of cinerenin, making the distinction between these two compounds impossible by pmr. Compound "2013" also shows pmr signals which overlap with the crucial isopropyl signals of 64, these signals are necessary for a positive identification of melampodin-C.

Consequently, the isolation of 64 by column chromatography appears to be the only reliable method for its detection.

5. Melampodin-B (56), a highly polar compound, usually crystallized from the crude syrups and provided extremely reliable confirmation. Melampodin-B also has the lowest rf values thereby permitting a somewhat reliable tlc analysis. Since 56 was the major component in most populations, it was the easiest to detect.

6. "2013" (73), a germacranolide whose structure elucidation is incomplete, shows rf values slightly higher than those of melampodin-C making tlc analysis unreliable. Compound "2013" shows characteristic H-13 signals at δ5.73 and δ6.14 and a CO_2Me signal at δ3.76 which could have made pmr detection possible if the spectra of the crude syrups had been clearly resolved. Unfortunately, most pmr spectra of the crude syrups did not provide data for conclusive identification.

In spite of the previously described experimental problems, the use of all available techniques provided reliable qualitative data that were very useful in the biochemical systematic analysis. The result of the populational study are summarized in Tables 11, 12, and 13.

TABLE 11

Column Chromatographic Analysis of M. cinereum and M. argophyllum*

Populational Number	Location	Amount	B	C	A	Other	Chromosome Number
2013	Duval Co., Tex.	345g	30mg	20mg	25mg	D,405mg F,100mg	20
2015B	Duval Co., Tex.	1450g	2.7g	4.0g	315mg		20
2016	Jim Hogg Co., Tex.	328g	303mg	+	+		20
2020	Tamaulipas, Mex.	750g	605mg	+	152mg	F, 35mg	10
2021	Hidalgo Co., Tex.	356g	650mg	30mg	+		10
3613	Jim Hogg Co., Tex.	719g	210mg	102mg	140mg		20
Fi29a	Live Oak Co., Tex.	200g	-	+	115mg	F,410mg	--
Fi29b	Live Oak Co., Tex.	389g	-	+	160mg	F, 58mg	--
3596*	Coahuila, Mex.	937g	450mg	308mg	685mg	E,175mg	--
3599*	Nuevo Leon, Mex.	1350g	3.1g	885mg	610mg	E,1.23g	30

A = Artemetin (78); B = Melampodin-B (56); C = Cinerenin (67)

D = "2013"; E = Melampodin-C (64); F = 4(5)-Dihydromelampodin-B (61)

TABLE 12

Populational Analysis of M. cinereum DC variety cinereum

Populational Number	Location	Amount	B	C	A	Other	Chromosome Number
2009	Live Oak Co., Tex.	126g	80mg	+	+		20
2010	Jim Wells Co., Tex.	102g	250mg	-	+		20
2011	Jim Wells, Co., Tex.	16g	5mg	-	+		20
2014	Duval Co., Tex.	191g	160mg	+	-		--
2015A	Duaval Co., Tex.	52g	43mg	+	+		20
2017	Brooks Co., Tex.	177g	180mg	+	+		20
2023	Starr Co., Tex.	47g	75mg	+	+	D	10
2024	Starr Co., Tex.	895g	235mg	+	+	D	20
2025	Zapata Co., Tex.	407g	185mg	+	+	D	20
2027	Zapata Co., Tex.	91g	34mg	+	+		20
2029	Webb Co., Tex.	422g	175mg	+	+	D	10
2030	Webb Co., Tex.	108g	25mg	+	+	D	10
2031	Dimmit Co., Tex.	66g	16mg	+	+	D	10
2032	Zavala Co., Tex.	158g	25mg	+	+	D	10

Table 12 (cont.)

Populational Number	Location	Amount	B	C	A	Other	Chromosome Number
2033	Uvalde Co., Tex.	404g	160mg	+	+	D	10
3588	Frio Co., Tex.	68g	156mg	+	+	D	10
3589	Frio Co., Tex.	146g	270mg	+	+	D	10
3589B	Frio Co., Tex.	12g	+	+	-		10
3593	Coahuila, Mex.	91g	+	+	+		10
3610	Webb Co., Tex.	134g	254mg	+	+		20
3611	Jim Hogg Co., Tex.	91g	375mg	+	+		20
3612	Jim Hogg Co., Tex.	95g	145mg	+	+		20
3614	Starr Co., Tex.	76g	250mg	+	+	D	10
3615	Jim Hogg Co., Tex.	43g	121mg	+	-	D	20
3616	Jim Hogg Co., Tex.	79g	139mg	+	+	D	20
3617	Jim Hogg Co., Tex.	85g	31mg	+	+	D	20
3618	Duval Co., Tex.	62g	5mg	+	+		20
3619	La Salle Co., Tex.	65g	20mg	+	+	D	10
3620	Frio Co., Tex.	678g	800mg	+	+		--

TABLE 13

Populational Analysis of M. cinereum DC variety hirtellum

Populational Number	Location	Amount	B	C	A	Other	Chromosome Number
2036	Kinney Co., Tex.	102g	120mg	+	+		20
3590	Maverick Co., Tex.	129g	98mg	+	+		10
3594	Coahuila, Mex.	46g	139mg	+	-		10
3597	Coahuila, Mex.	38g	+	+	-	D	10
3605	Nuevo Leon, Mex.	61g	142mg	+	-		10
3606	Nuevo Leon, Mex.	42g	165mg	+	-		10
3608	Nuevo Leon, Mex.	66g	61mg	+	-		10
3609	Nuevo Leon, Mex.	29g	42mg	+	-		10
2018*	Tamaulipas, Mex.	147g	15mg	-	+		--
3603**	Nuevo Leon, Mex.	103g	551mg	+	+		30

* Melampodium cinereum var ramosissimum

** Melampodium argophyllum

A = Artemetin
B = Melampodin-B (56)
C = Cinerenin (67)
D = "2013"

The data compiled in this study clearly indicate that the consistently occurring melampodin-B (56), cinerenin (67), and artemetin (78) are characteristic constituents of M. cinereum. The quantitative populational variations of sesquiterpene lactones might have been influenced by a number of factors. The stage of growth as well as environmental conditions such as elevation, soil, water levels, and the dark-light cycle are known to influence the relative amounts of secondary plant metabolites. The presence of 4(5)-dihydromelampodin-B (61) and the lack of melampodin-B in one population of M. cinereum (Fischer #29) seems to be indicative of an active reducing enzyme in this particular population.

The presence or absence of the compound "2013" in other populations of M. cinereum will have to be more carefully determined. Tedious chromatographic separations of the plant constituents in each population might be necessary before definite qualitative evaluation concerning compound "2013" can be made. Furthermore, completion of the structure elucidation of compound "2013" will appreciate the value of this compound in the biochemical systematic considerations of the white-rayed complex of Melampodium.

In spite of the limited number of chemically investigated populations of M. argophyllum, a general trend can be recognized. On the basis of the sesquiterpene lactone constituents, M. argophyllum seems to be very closely related to M. cinereum. All constituents typical of the latter species are also found in M. argophyllum. In addition, melampodin-C (64) is present in M. argophyllum but has not yet been found in M. cinereum. It would

be premature, however, to recognize melampodin-C as a typical marker of M. argophyllum (to date, 64 exclusively occurs in this species). A large number of populations need to be chemically analyzed before more definite conclusions can be drawn.

In general, no major qualitative differences were recognized between the different populations of M. cinereum and M. argophyllum indicating considerable stability in the evolutionary process of the different varieties of these species. In contrast, M. leucanthum represents a chemically more diversified and evolutionarily more dynamic species. Consequently, it might be hypothesized that the above data indicate the existence of a more advanced, stable evolutionary state in M. cinereum and M. argophyllum.

The populational analysis of the sesquiterpene lactones in M. cinereum and M. argophyllum is not completed; however, the following tentative conclusion can be drawn from the initial data: on the basis of the structural similarity of the sesquiterpene lactones in these two species, and the fact that these sesquiterpene lactones are distinctly different from those in M. leucanthum, it appears that, in contrast to Stuessy's morphological considerations, M. argophyllum is phylogenetically more closely related to M. cinereum than it is to M. leucanthum.

PART III

Experimental

EXPERIMENTAL

The following instruments were used in obtaining the various data throughout this dissertation unless otherwise specifically indicated:

IR Perkin-Elmer 137 Sodium Chloride Spectrophotometer

UV Cary-14 Recording Spectrophotometer

CD Durram-Jasco J-20 Spectrometer

MP Thomas Hoover Capillary Melting Point Apparatus (uncorrected)

60 MHz NMR Perkin-Elmer R12B Spectrometer

100 MHz NMR Varian HA-100 Spectrometer

Mass Spectra Hitachi-Perkin-Elmer Model RMS-4 (samples were introduced *via* the direct inlet tube)

Elemental Analyses Galbraith Laboratories, Inc., Knoxville, Tennessee U.S.A.

Isolation of Melampodin-B (56) and Cinerenin (67)

A collection of M. cinereum DC. var. cinereum was made on July 19, 1973 (T. F. Stuessy and N. H. Fischer 2015) 8.6 miles north-east of Hebbronville, Duval County, Texas on route 359.

Dried leaves (1475 g.) were ground in a Waring blender with cold chloroform. After filtration by suction and re-extraction of the residue, the combined $CHCl_3$ extracts were evaporated in vacuo. The dark residue was dissolved in 1450 ml of ethanol and then the same volume of an aqueous 5% lead(II)acetate solution was added. The gummy precipitate was removed by filtration over celite and the filtrate evaporated in vacuo to remove most of the ethanol. The residual aqueous phase was extracted exhaustively with $CHCl_3$. After drying with anhydrous $MgSO_4$, removal of the solvent left the crude, terpenoid-containing syrup. The syrup was allowed to stant at room temperature for several days resulting in a partial crystallization of the syrup. Filtration and washing of the residue with ether gave a yellow crystalline solid. Repeated trituration of this material with hot ethyl acetate left 2.7 g of crude melampodin-B (56), mp 226-228° (dec.); UV, λ_{max}^{MeOH} 204 nm (ϵ, 2.35×10^4); CD (c, 6.2×10^{-5}, MeOH), $[\theta]_{216}$ -8.3×10^3, $[\theta]_{238}$ $+5.4 \times 10^3$, $[\theta]_{277}$ -5.4×10^2; IR, ν_{max} (nujol) 3420 (OH), 1780 (γ-lactone), 1730 (α,β-unsat. ester), and 1665 cm^{-1} (double bonds). NMR data of 56 listed in Tables 1 and 2. Mass Spectral data of 56 listed in Table 14.

Anal. Calcd. for $C_{17}H_{18}O_7$: C, 61.07; H, 5.43; O, 33.50; MW, 334 Found: C, 60.95; H, 5.45; O, 33.76.

The combined ethyl acetate extracts provided a final yield of 4.0 g of crude cinerenin (67). Analytically pure 67 was obtained after 100mg of crude cinerenin was chromatographed on a silica gel column using as eluting solvent a mixture of CH_2Cl_2EtOAc (4:1). The crude 67 was recrystallized from EtOAc giving colorless crystals: mp 161-163; UV, $\lambda^{MeOH}_{end.\ abs.}$ 205 nm (ϵ, 2.74 x 10^4); CD, (c, 6.25 x 10^{-5}, MeOH), $[\theta]_{218}$ - 4.1 x 10^4, $[\theta]_{240}$ + 4.7 x 10^4, $[\theta]_{280}$ - 6.6 x 10^2; IR, ν_{max} (nujol) 3450 broad (OH), 1775 (γ-lactone), 1750 (α,β-unsat. ester), and 1660 cm^{-1} (double bonds). NMR data of 67 listed in Table 7. Mass Spectral data of 67 listed in Table 16.

Anal. Calcd. for $C_{17}H_{20}O_6$: C, 63.74; H, 6.29; MW, 320. Found: C, 63.54; H, 6.20; MW(MS), 320.

Melampodin-B Acetate (57)

A solution of 56 (103mg) was heated in 1ml of pyridine and 1ml of Ac_2O until all of the crystals were dissolved. The solution was left overnight at room temperature. Evaporation under reduced pressure provided a white residue. Water (5ml) and a drop of HCl were added and the slurry extracted three times with 50ml portions of $CHCl_3$. The combined $CHCl_3$ extracts were dried ($MgSO_4$), filtered and evaporated. The residual white powder was recrystallized from EtOAc providing 70 mg of 57: mp 202-204°; UV, λ^{MeOH}_{max} 206 nm (ϵ, 3.0 x 10^4); CD, (c, 7.1 x 10^{-4}, MeOH), $[\theta]_{216}$ - 2.9 x 10^4, $[\theta]_{233}$ + 4.5 x 10^4; IR, ν_{max} (nujol) 1790, 1770 (γ-lactones), 1735, 1230 (acetate), and 1660 cm^{-1} (double bonds). NMR data of 57 listed in Table 3. Mass Spectral data of 57 listed in Table 14.

Anal. Calcd. for $C_{19}H_{20}O_8$: C, 60.64; H, 5.32; MW, 376. Found: C, 60.55; H, 5.41; MW(MS), 376.

Melampodin-B Aldehyde (58)

Melampodin-B (100 mg) in 50 ml of acetone under nitrogen was treated with an excess of Sarett's reagent[79] in acetone. After 4 hours the brown precipitate was filtered and the acetone evaporated. Water (5 ml) was added and the slurry twice extracted with 75 ml of $CHCl_3$ and once with 50 ml of EtOAc. The combined organic extracts were dried over $MgSO_4$, filtered and evaporated. The impure brownish crystals were dissolved in acetone and chromatographed over silica gel/CH_2Cl_2. The column was eluted with CH_2Cl_2, CH_2Cl_2/EtOAc (1:1), and finally with pure EtOAc. The recovered crystals were recrystallized from acetone/2-propanol providing 45 mg of colorless, crystalline 58: mp 241-245° (dec.); UV, λ_{max}^{MeOH}215 nm (ε, 3.2 x 10^4); IR, ν_{max} (nujol) 1775 (γ-lactone), 1740 (acetate), 1700, 1660 (double bonds), and 1240 cm^{-1}. NMR data of 58 listed in Table 3.

Anal. Calcd. for $C_{17}H_{16}O_7$: C, 61.45; H, 4.82; MW, 332. Found: C, 61.26; H, 4.84; MW(MS) 332.

11(13)-Dihydromelampodin-B (59)

A solution of 100 mg of melampodin-B in 90 ml of MeOH was added to 5 mg of 10% Pd/C and placed in a 100 ml three-necked flask. After removing the residual air in vacuo, H_2 was allowed to react with the stirred mixture. Initial uptake of H_2 was rapid for

about 15 min. with the reaction terminating in approximately one hour. After filtration and evaporation, the residue was chromatographed on a silica gel column using EtOAc as an elutant. The fractions were analyzed by tlc and combined appropriately. The crude residue was recrystallized from EtOAc providing 30 mg of 11(13)-dihydromelampodin-B: mp 200-202°; IR, ν_{max} (nujol) 3420 (OH), 1775 (γ-lactone), 1735 and 1245 cm^{-1} (acetate). NMR data of 59 listed in Table 3.

Anal. Calcd. for $C_{17}H_{20}O_7$: C, 60.71; H, 5.99; O, 33.30; MW 336. Found: C, 60.90; H, 5.95.

Tribromide (60) from Melampodin-B (56)

A solution of melampodin-B (100 mg) in 25 ml glacial acetic and 3.0 ml of a saturated solution of HBr in acetic acid was refluxed for 20 hrs. The solvent was evaporated and ethyl ether was added which resulted in the precipitation of a white solid. Recrystallization from 2-propanol yielded 85 mg of pure 60: mp 215-217°; IR, ν_{max} (nujol) 1775 (γ-lactone), 1650 (double bonds), 1200, and 1000 cm^{-1}. NMR data of 60 listed in Table 3.

Anal. Calcd. for $C_{15}H_{15}O_4Br_3$: C, 36.06; H, 3.01; Br, 48.10; MW 499. Found: C, 36.16; H, 3.07; Br, 48.00; MW(MS) 497-501.

Reaction of Melampodin-B (56) with Lead(II)acetate

100mg of 56 was stirred overnight at room temperature in a mixture of 50 ml of 5% lead(II)acetate in H_2O and 50 ml of EtOH.

The solution was filtered and the filtrate evaporated to approximately 30 ml which was then extracted three times with 30 ml each of $CHCl_3$. After drying with $MgSO_4$, the combined $CHCl_3$ extracts were filtered and the solvent evaporated. A crude white residue was left, which after recrystallization from acetone gave unchanged 56 characterized by mp, mixture melting point, ir, and pmr with authentic material.

Isolation of 4(5)-Dihydromelampodin-B (61)

A collection of M. cinereum, var. cinereum was first made on October 4, 1971 (N. H. Fischer #12) 12 miles south of George West, Texas on Highway 59 and again on July 7, 1973 (N. H. Fischer #29). NMR spectra of the crude extracts indicated the presence of the same constituents of the previously described collections.

The dried leaves (200 g) were extracted and worked up as illustrated in Scheme IX providing 18 g of crude terpenoid-containing syrup. The crude syrup (9 g) was chromatographed over 300 grams of silica gel (Baker 3405) collecting 15 ml fractions and using the following solvent mixtures: fraction 1-40 (CH_2Cl_2/EtOAc, 9:1); 41-60 (CH_2Cl_2/EtOAc, 4:1); 61-100 (CH_2Cl_2/EtOAc, 3:1); 100-170 (CH_2Cl_2/EtOAc, 1:1); 171-190 (CH_2Cl_2/EtOAc, 1:4); 191-220 (pure EtOAc); 221-260 (5% MeOH in EtOAc); fractions 261-289 (MeOH/EtOAc, 1:1). The following fractions were collected and combined according to tlc analysis: fractions 40-57 gave 50 mg of artemetin, mp 159-160°, identical with an authentic sample* by mixture mp and spectral

*Supplied by Werner Herz, Florida State University

comparison (pmr, ir). Fractions 227-261 provided 300 mg of 4(5)-dihydromelampodin-B (61): mp 204-205°; UV, λ_{max}^{EtOH} 202 nm (ϵ, 3.2 x 10^4); CD, (c, 7.77 x 10^{-5}, MeOH), $[\theta]_{220}$ - 106 x 10^3; $[\theta]_{265}$ - 1.6 x 10^3; IR, ν_{max} (nujol) 3400 (OH), 1785 (γ-lactone), 1750 (acetate), and 1665 cm^{-1} (double bonds). NMR data of 61 listed in Table 4. Mass Spectral data of 61 listed in Table 14.

Anal. Calcd. for $C_{17}H_{20}O_7$: C, 60.71; H, 5.99; MW 336. Found: C, 61.01; H, 6.31; MW(MS) 336.

4(5)-Dihydromelampodin-B Acetate (62)

A solution of 100 mg of 61 in 1 ml of pyridine and 1 ml of Ac_2O was left overnight at room temperature. Evaporation of the solution in vacuo left a yellow-white residue. Water (5 ml) and a drop of conc. HCl were added and the slurry was extracted three times with 25 ml of $CHCl_3$ and the $CHCl_3$ extract dried over $MgSO_4$. After filtration, the $CHCl_3$ was evaporated leaving a colorless residue. Recrystallization from EtOAc/Et_2O provided 95 mg of 62: mp 195-196°; IR, ν_{max} (nujol) 1770 (γ-lactone), 1680, 1245 (acetate), and 1070 cm^{-1}. NMR data of 62 listed in Table 4. Mass Spectral data of 62 listed in Table 14.

Anal. Calcd. for $C_{19}H_{22}O_8$: C, 60.31, H, 5.86; O, 33.83; MW 378. Found: C, 60.60; H, 5.80; O, 33.73; MW(MS) 378.

4(5),11(13)-Tetrahydromelampodin-B (63)

A solution of 100 mg of 4(5)-dihydromelampodin-B (61) in 75 ml of MeOH and 5 mg of 10% Pd/C were placed in a 100 ml three-necked flask. After removal of air in vacuo the stirred mixture was hydrogenated resulting in the rapid uptake (15 min.) of hydrogen with the reaction terminating after about one hour. Filtration and evaporation provided a syrup which was chromatographed by plc using propyl acetate as developing solvent. The band at rf 0.3 was extracted from the silica gel and the resulting crude material was recrystallized from acetone giving 35 mg of 63: mp 220-222°; IR, ν_{max} (nujol) 3500 (OH), 1770, 1750 (γ-lactone), 1725, and 1240 cm^{-1} (acetate). NMR data of 63 listed in Table 4.

Anal. Calcd. for $C_{17}H_{22}O_7$: C, 60.34; H, 6.55; MW 338. Found: C, 60.42; H, 6.27.

Isolation of Melampodin-C (64)

A collection of M. argophyllum was made on July 3, 1974 (T. F. Stuessy 3599) 30 miles southeast of the Coahuila-Nuevo Leon border on route 53 in the state of Nuevo Leon, Mexico.

Dried leaves (1350 g) were extracted and worked up as described before (Scheme IX). The crude syrup (22 g), when allowed to stand at room temperature for three weeks, partially crystallized providing 3.0 g of crude melampodin-B (56). The remaining crude syrup (10 g) was chromatographed over 300 g of silica gel (Brinkmann 7734) collecting 20 ml fractions. The column was eluted using the following

solvent mixtures: 1000 ml of CH_2Cl_2/EtOAc (9:1); 700 ml of CH_2Cl_2/EtOAc (1:1); 300 ml of EtOAc and 500 ml of 15% MeOH in EtOAc. The following fractions were collected and combined according to tlc analysis: fractions 21-40 contained 610 mg of artemetin. Fractions 71-100 provided 1.23 g of melampodin-C (64). Recrystallization from EtOAc/Et_2O gave colorless crystals: mp 199-201°; UV, $\lambda^{MeOH}_{end.abs.}$ 205 nm (ε, 2.91 x 10^4); CD, (c, 5.5 x 10^{-5}, MeOH), $[\theta]_{216}$ - 8.3 x 10^3, $[\theta]_{138}$ + 5.4 x 10^3, $[\theta]_{280}$ - 9.3 x 10^2; IR, ν_{max} (nujol) 3500 (OH), 1770 (γ-lactone), 1725 (ester), and 1655 cm^{-1}. NMR data of 64 listed in Table 5. Mass Spectral data of 64 listed in Table 15.

Anal. Calcd. for $C_{19}H_{22}O_7$: C, 62.97; H, 6.12; O, 30.91; MW, 362. Found: C, 63.07; H, 6.00; O, 30.77; MW(MS) 362). Fractions 116-130 gave 890 mg of cinerenin (67) and 3.0 g of melampodin-B (56) was present in fractions 140-160.

Melampodin-C Acetate (65)

A solution of 170 mg of 64 in 2 ml of pyridine and 3 ml of Ac_2O was heated until all the crystals were dissolved. The solution was left overnight at room temperature. Evaporation in vacuo provided a white residue to which 5 ml of H_2O and 5 drops of HCl were added. The slurry was extracted three times with 50 ml portions of CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$, filtered, and evaporated leaving a white residue which was recrystallized from 2-propanol providing 160 mg of 65: mp 151-153°; IR, ν_{max} (nujol) 1780, 1765 (γ-lactone), and 1225 (acetate). NMR

data of 65 listed in Table 5. Mass Spectral data of 65 listed in Table 15.

Anal. Calcd. for $C_{21}H_{24}O_8$: C, 62.37; H, 5.98; O, 31.65; MW 404. Found: C, 62.53; H, 5.96; O, 31.57; MW(MS) 404.

Melampodin-C Aldehyde (66)

To a solution of 64 (150 mg) in 25 ml of acetone under nitrogen was added an excess of Sarett's reagent,[79] suspended in acetone. After 4 hours, the brown precipitate was filtered and the acetone was evaporated. The crude residue was chromatographed over 25 g of silica gel. The column was eluted with 300 ml of CH_2Cl_2/EtOAc (9:1), then with 200 ml of CH_2Cl_2/EtOAc (1:1). Evaporation of all fractions gave a white powder which upon recrystallization from EtOAc provided 71 mg of the pure aldehyde 66: mp 207-209°; IR, ν_{max} (nujol) 1785 (γ-lactone), 1735 (ester) 1700, 1245, and 1145 cm^{-1}. NMR data of 66 listed in Table 5. Mass Spectral data of 66 listed in Table 15.

Anal. Calcd. for $C_{19}H_{20}O_7$: C, 63.33; H, 5.59; O, 31.08; MW 360. Found: C, 63.46; H, 5.64; O, 30.84; MW(MS) 360.

Tribromide 60 from Melampodin-C (64)

A solution of 64 (100 mg) in 25 ml of EtOAc and 3 ml of a saturated solution of HBr in glacial acetic acid was refluxed for 20 hours. The solvent was evaporated and after the addition of ethyl ether the resulting white precipitate was recrystallized from 2-propanol providing 60 mg of crystalline 60, identical with the

material from melampodin-B, and cinerenin by mp, mixture melting point, and spectral comparison (IR and PMR).

Cinerenin Acetate (68)

A solution of 100 mg of 67 in 1 ml of pyridine and 1 ml of Ac_2O was left overnight at room temperature. Evaporation of the mixture *in vacuo* left a yellow-white residue. Water (5 ml) and a drop of conc. HCl were added and the slurry was extracted three times with 25 ml portions of $CHCl_3$. The combined $CHCl_3$ extracts were dried over $MgSO_4$. After filtration the $CHCl_3$ was evaporated leaving a white powder which upon recrystallization from 2-propanol gave 60 mg of pure 68: mp 187-189°; IR, ν_{max} (nujol) 1780, 1765 (γ-lactone), 1730, 1235, 1225 (acetate), and 1665 cm^{-1} (double bonds). NMR data of 68 listed in Table 8. Mass Spectral data of 68 listed in Table 16.

Anal. Calcd. for $C_{19}H_{22}O_7$: C, 62.97; H, 6.12; MW, 362. Found: C, 62.89; H, 6.11; MW(MS), 362.

Cinerenin Aldehyde (69)

To a solution of 105 mg of 67 in 25 ml of acetone in a nitrogen atmosphere was added an excess of Sarett's reagent,[79] suspended in acetone. After four hours, the brown precipitate was filtered and the acetone evaporated. Water (5 ml) was added and the slurry extracted three times with 50 ml portions of $CHCl_3$. The combined organic extracts were dried ($MgSO_4$) and evaporated. The crude

brownish crystals were taken up in acetone and chromatographed over silica gel. The column was eluted with CH_2Cl_2, CH_2Cl_2/EtOAc (1:1), and finally with pure EtOAc which upon appropriate combination gave colorless crystals. Recrystallization from 2-propanol/chloroform gave 50 mg of pure 69: mp 224-225°; UV, λ_{max}^{MeOH} 216 nm (ε, 2.7 x 10^4); IR, ν_{max} (nujol) 1770 (γ-lactone), 1700 (ester), and 1650 cm^{-1}. NMR data of 69 listed in Table 8. Mass Spectral data of 69 listed in Table 16.

Anal. Calcd. for $C_{17}H_{18}O_6$: C, 64.14; H, 5.70; O, 30.16; MW, 218. Found: C, 63.93; H, 5.59; O, 30.35; MW(MS), 318.

Tribromide 60 from Cinerenin (67)

The conditions and results of this experiment were identical to those of the analogous reactions with melampodin-B and Melamodin-C. All three compounds (56, 64, and 67) gave the tribromo compound 60.

Isolation of Melampodinin (70)

Bulk collection of Melampodium americanum were made during the summer of 1974 in central Mexico. (T. F. Stuessy, #3171)

Dried leaves (1012 g.) were extracted and worked-up as previously described (Scheme IX) providing 2.5 g of crude, terpenoid containing syrup. Chromatography over 100 g of silica gel using CH_2Cl_2/EtOAc (9:1) as elutant; 15 ml fractions were taken and all fractions were monitored by tlc. Fractions 11-20 contained about

3.0 mg of artemetin (78) which was identical with authentic material by mp, and mixed-melting point. Fractions 36-60 provided 75 mg of 70: mp, 203-205°; UV, λ_{max}^{MeOH} 213 nm (ε, 1.70 x 10^4); CD, (c, 3.8 x 10^{-5}, MeOH), $[\theta]_{213}$ - 1.5 x 10^5, $[\theta]_{246}$ + 3.2 x 10^4; IR, ν_{max} (nujol) 3500 (OH), 1770 (γ-lactone), 1730 (ester), 1670, and 1650 cm^{-1} (double bonds). NMR data of 70 listed in Table 9.

Anal. Calcd. for $C_{25}H_{30}O_{12}$: C, 57.47; H, 5.79; MW, 522. Found: C, 57.51; H, 5.80; MW(MS),-

TABLE 14

Mass Spectral Data

of

Melampodin-B(56) and 4(5)-Dihydromelampodin-B(61)

and their Derivatives

56		57		61		62	
m/e	Relative Intensity	m/e	Relative Intensity	m/e	Relative Intensity	m/e	Relative Intensity
274	53.5	376	21.8	336	25.4	378	81.3
256	100	333	26.5	276	19.8	335	18.8
245	34.9	274	97.0	248	33.2	276	70.0
228	96.5	256	100	230	67.0	258	100
227	58.1	228	93.0	228	36.8	230	81.3
210	62.5	165	62.8	201	30.2	165	62.5
165	68.6	162	22.6	165	35.3	162	86.4
162	30.0	147	58.8	162	49.5	149	100
91	69.9	91	80.4	91	49.5	111	129
43	51.2	43	(943)	43	100	91	113
MW	334	MW	376	MW	336	43	(2240)
						MW	378

TABLE 15

Mass Spectral Data

of

Melampodin-C (64) and Derivatives

64		65		66	
m/e	Relative Intensity	m/e	Relative Intensity	m/e	Relative Intensity
362	0.34	404	3.33	360	0.72
344	0.17	361	1.51	359	1.08
274	9.90	317	8.80	345	1.44
256	6.82	274	12.4	344	2.16
228	7.61	256	14.5	329	1.80
227	3.30	228	11.6	315	3.96
165	5.12	165	7.90	274	1.26
147	4.78	147	6.05	257	7.55
91	11.8	91	6.36	230	12.2
88	18.2	71	39.0	212	18.0
73	38.6	43	100	91	18.0
43	100	MW	404	71	56.5
39	56.8			71	31.6
MW	362			57	61.7
				43	46.7
				29	100
				MW	360

TABLE 16

Mass Spectral Data

of

Cinerenin (67) and Derivatives

67		68		69	
m/e	Relative Intensity	m/e	Relative Intensity	m/e	Relative Intensity
320	3.14	362	3.27	318	1.77
274	50.0	333	1.30	317	3.03
256	70.5	318	3.21	303	1.51
228	58.0	316	18.8	300	1.51
227	37.6	274	100	290	7.50
199	36.0	256	61.7	274	44.4
179	37.6	228	70.4	256	16.5
165	67.2	178	32.7	228	9.30
147	70.5	165	61.7	178	63.0
112	100	162	28.8	165	24.0
91	50.0	147	36.1	112	100
43	18.2	112	38.8	91	42.2
MW	320	43	67.2	29	71.3
		MW	362	MW	318

TABLE 17

Collection Sites of the Various Populations of Melampodium

Species Identification*	Abbreviation
M. cinereum variety cinereum	M.cin.cin.
M. cinereum variety hirtellum	M.cin.hirt.
M. cinereum variety ramosissimum	M.cin.ram.
M. argophyllum	M.argophm.

Pop. #	Species	Date	Location
2009	M.cin.cin.	July 10, 1973	Live Oak Co., Texas (7.4 mi. S. of George West, jct. rtes. 281 & 59 on rte. 281)
2010	M.cin.cin.	July 19, 1973	Jim Wells Co., Texas (30.7 mi. S. of George West, jct. rtes. 281 & 59 on rte. 281. 10.2 mi. N. of jct. 281 & 44 in Alice)
2011	M.cin.cin.	July 19, 1973	Jim Wells Co., Texas (8.0 mi. W. of Alice; jct. rtes. 281 & 44 on 44)
2013	M.cin.cin.	July 19, 1973	Duval Co., Texas (1.4 mi. SW of San Diego; jct. rtes. 359 & 44 on 44)
2014	M.cin.cin.	July 19, 1973	Duval Co., Texas (7.1 mi. SW of Benavides; jct. rtes. 339 (north fork) & 359 on rte. 359)
2015A 2015B	M.cin.cin.	July 19, 1973	Duval Co., Texas (4.4 mi. SW of Realitos: jct. rtes. 359 & farm rd, 716 on rte. 359; 8.6 mi. NE of Hebbronville)
2016	M.cin.cin.	July 19, 1973	Jim Hogg Co., Texas (2.3 mi. W. jct. 16 on farm rd. 3073)
2017	M.cin.cin.	July 19, 1973	Brooks Co., Texas (12.4 mi. E of Hebbronville; jct. rte. 16 on rte. 285)

*by Dr. Tod F. Stuessy, Dept. of Botany, Ohio State University

TABLE 17 (cont.)

Pop. #	Species	Date	Location
2018	M.cin.ram.	July 20, 1973	Tamaulipas, Mexico (3.8 mi. E jct. rte. 97 on rte. 2; 5 mi. SW of Reynosa)
2020	M.cin.ram.	July 20, 1973	Tamaulipas, Mexico (2.6 mi. S of jct. rte. 2 on rte. 97; km 109.5)
2021	M.cin.cin.	July 21, 1973	Hidalgo Co., Texas (0.8 mi. E of jct. with farm rd. 886; 1.8 mi. E of Sullivan City on rte. 83)
2023	M.cin.cin.	July 21, 1973	Starr Co., Texas (0.2 mi. W jct. farm rd. 1430 on rte. 83)
2024	M.cin.cin.	July 21, 1973	Starr Co., Texas (4.5 mi. W jct. farm rd. 649 on rte. 83)
2025	M.cin.cin.	July 21, 1973	Zapata Co., Texas (0.2 mi. SSE of city limits of Lopeno on rte. 83)
2027	M.cin.cin.	July 21, 1973	Zapata Co., Texas (3.8 mi. NW of San Ygnacio; jct. rte. 83 & farm rd. 3169)
2029	M.cin.cin.	July 22, 1973	Webb Co., Texas (1.8 mi. NNW jct. rte. 35 on rte 83)
2030	M.cin.cin.	July 22, 1973	Webb Co., Texas (1.0 mi. E of jct. rtes. 44 & 83)
2031	M.cin.cin.	July 22, 1973	Dimmit Co., Texas (6.3 mi. N of Carrizo Springs; jct. rtes. 83 & 85 on rte. 83)
2032	M.cin.cin.	July 22, 1973	Zayala Co., Texas (11.9 mi. S of Uvalde; jct. rte. 90 on rte. 83; c.a. 0.5 mi. S of Nueces river bridge)
2033	M.cin.cin.	July 22, 1973	Uvalde Co., Texas (8.9 mi. S of Uvalde; jct. rte. 90 on rte. 83)
3605	M.cin.hirt.	July 3, 1974	Nuevo Leon, Mexico (jct. rtes. 53 & 85; c.a. 10 mi. N of Monterrey)
3606	M.cin.hirt.	July 3, 1974	Nuevo Leon, Mexico (43 mi. N of jct. 53 on rte. 85; c.a. 63 mi. N of Monterrey)

TABLE 17 (cont.)

Pop. #	Species	Date	Location
3608	M.cin.hirt.	July 3, 1974	Nuevo Leon, Mexico (11.7 mi. N of Sabinas Hidalgo, at N Pemex Station on rte. 85)
3609	M.cin.hirt.	July 3, 1974	Nuevo Leon, Mexico (33.2 mi N of Sabinas Hidalgo at N Pemex Station on rte. 85)
3610	M.cin.cin.	July 4, 1974	Webb Co., Texas (20.5 mi. E of Laredo jct. rtes. 83 & 359)
3611	M.cin.cin.	July 4, 1974	Jim Hogg Co., Texas (0.3 mi. S jct. farm rd. 649 and 2073; 15.8 mi. S of Miranda City)
3612	M.cin.cin.	July 4, 1974	Jim Hogg Co., Texas (8.4 mi. S of jct. rte. 16 on farm rd 649)
3613	M.cin.cin.	July 4, 1974	Jim Hogg Co., Texas (7.9 mi. N of jct. farm rd. 2686 on farm rd. 649)
3614	M.cin.cin.	July 4, 1974	Starr Co., Texas (2.8 mi. N of El Sauz on farm rd. 649)
3615	M.cin.cin.	July 5, 1974	Jim Hogg Co., Texas (10.6 mi. N of jct. farm rd. 2686 on farm rd. 1017; 4.9 mi. S of Aqua Nuevo)
3616	M.cin.cin.	July 5, 1974	Jim Hogg Co., Texas (20.8 mi. N of Aqua Nueva on farm rd. 1017)
3617	M.cin.cin.	July 5, 1974	Jim Hogg Co., Texas (1.5 mi. N of Hebbronville jct. rte. 285 on rte. 16)
2036	M.cin.hirt.	July 22, 1974	Kinney Co., Texas (10.9 mi. W of Brackettville; jct. farm rd. 131 on rte. 90)
3588	M.cin.cin.	June 30, 1974	Frio Co., Texas (7.3 mi. S of jct. farm rd. 479 on farm rd. 2774; c.a. 8 mi. S of Moore)
3589 3589B	M.cin.cin.	June 30, 1974	Frio Co., Texas (1.7 mi. W of Divot; jct. farm rd. 1581 on W farm rd. 117)

TABLE 17 (cont.)

Pop. #	Species	Date	Location
3590	M.cin.hirt.	July 1, 1974	Maverick Co., Texas (7.1 mi. S of jct. farm rd. 375 on farm rd. 1021; c.a. 8 mi. S of Eagle Pass)
3593	M.cin.cin.	July 2, 1974	Coahuila, Mexico (18.6 mi. S of Allende on rte. 57; 0.4 mi. N of km 59)
3594	M.cin.hirt.	July 2, 1974	Coahuila, Mexico (3.0 mi. W of San Juan de Sabrinas on rte. 53)
3596	M.argo.	July 2, 1974	Coahuila, Mexico (Rancho Pajaros Azules, 11.9 mi. SE of Tujillo; 7 mi. SE of Ejido Primero de Mayo which is on rte. 57; on mountain to SW of ranch house, then up foothills in limestone rock)
3597	M.cin.hirt.	July 2, 1974	Coahuila, Mexico (22.9 mi. S of Monclova across from steel works at S end of town on rte. 57)
3599	M.argo.	July 3, 1974	Nuevo Leon, Mexico (29.7 mi. SE of Coahuila-Nuevo Leon border on rte. 53; hills N of the Microondas Pedernales station)
3603	M.argo.	July 3, 1974	Nuevo Leon, Mexico (31.8 mi. SE of Coahuila-Nuevo Leon border on rte. 53; 2.1 mi. SE of Microondas Pedernales station on sloping rd. shoulders)
3618	M.cin.cin.	July 5, 1974	Duval Co., Texas (36.3 mi. N of Hebbronville; jct. rte. 285 on rte. 16)
3619	M.cin.cin.	July 5, 1974	LaSalle Co., Texas (0.4 mi. S of Nucces River just S of Cortulla on US 81)
3620	M.cin.cin.	July 5, 1974	Frio Co., Texas (1.7 mi. W of Divot; jct. farm rd. 1581 on farm rd. 117, same as 3589)
F129a	M.cin.cin.	October 4, 1971	Live Oak Co., Texas (12 mi. S of George West on rte. 59)
F129b	M.cin.cin.	July 7, 1973	Live Oak Co., Texas (12 mi. S of George West rte. 59)

REFERENCES

1. T. A. Geissman, and D. H. Crout, Organic Chemistry of Secondary Plant Metabolism, (Freeman, Cooper, & Co., San Francisco, Calif., 1969) p. 5.

2. R. E. Alston and B. L. Turner, Biochemical Systematics, (Prentice-Hall, Englewood Cliffs, New Jersey, 1963).

3. R. E. Alston, Evolutionary Biology I, (Meredith Publishing Co., New York, N. Y., 1967) pp. 197-305.

4. B. L. Turner, Taxon, 18, 134 (1969).

5. V. Herout, and F. Sorm, Perspectives in Phytochemistry, Edited by J. B. Harbonrne and T. Swain, (Academic Press, London, 1969), pp. 139-165.

6. S. M. Kupchan, J. C. Hemingway, J. M. Cassady, and J. R. Knox, J. Amer. Chem. Soc. 89, 465 (1967).

7. S. M. Kupchan, M. A. Eakin, and A. M. Thomas, J. Med. Chem., 14, 1147 (1971).

8. N. H. Fischer, T. J. Mabry, and H. B. Kagan, Tetrahedron, 24, 4091 (1968).

9. H. Yoshioka, W. Renold, N. H. Fischer, A. Higo, and T. J. Mabry, Phytochemistry 9, 823, (1970).

10. L. Novotny, J. Jizba, V. Herout, and F. Sorm, Coll. Czech. Chem. Commun., 27, 1393 (1962).

11. M. A. Irwin and T. A. Geissman, Phytochemistry, 12, 863 (1973).

12. N. H. Fischer and T. J. Mabry, Tetrahedron, 23, 2529 (1967).

13. L. Ruzicka, Pure Appl. Chem., 6, 493 (1963).

14. J. B. Hendrickson, Tetrahedron, 7, 82 (1959).

15. W. J. Parker, S. Roberts, and R. Ramage, Quart, Rev., 3., 331 (1967).

16. E. E. Waldner, C. Schlatter, and H. Schmidt, Helv. Chim. Acta., 52, 515 (1969).

17. A. J. Birch and S. F. Hussein, J. Chem. Soc., C, 1969, 1473.

18. M. Anchel, T. C. McMorris, and P. Singh, Phytochemistry, 9, 2339 (1970).

19. J. Stauton, Annu. Rep. Chem. Soc., 1969, 555 1969.

20. E. E. van Tamelen, Accounts Chem. Res., 1, 111 (1968).

21. T. A. Geissman, "The Biogenesis of Sesquiterpene Lactones of the Compositae", in Terpenoids: Structure, Biogenesis, and Distribution, Ed. by T. J. Mabry and V. C. Runeckles, (Academic Press, New York & London, 1973), pp. 65-95.

22. H. Rees, L. J. Goad, and T. W. Goodwin, Tetrahedron Lett., 723 (1968).

23. S. M. Kupchan, J. E. Kelsey, and G. A. Sims, Tetrahedron Lett., 2863 (1967).

24. H. Yoshioka, T. J. Mabry, and B. N. Timmermann, Sesquiterpene Lactones, (University of Tokyo Press, 1973), pp. 3-114.

25. J. P. Herdrickson, Tetrahedron, 19, 1387 (1963).

26. A. J. Weinheimer, W. W. Youngblood, P. H. Washecheck, T. B. Karns, and L. S. Ciereszko, Tetrahedron Lett., 497 (1970).

27. N. H. Fischer, R. Wiley, and J. D. Wander, J.C.S. Chem. Commun., 1972, 137.

28. S. Neidle and D. Rogers, J.C.S. Chem. Commun., 1970, 140.

29. I. Bernal and S. F. Watkins, Science, 178, 1282 (1972).

30. D. Rogers, G. P. Moss, and S. Neidle, J.C.S. Chem. Commun., 1970, 142.

31. S. F. Watkins, N. H. Fischer, and I. Bernal, Proc. Nat. Acad. Sci. USA., 70, 2434 (1973).

32. N. S. Bhacca, and N. H. Fischer, J.C.S. Chem. Commun., 1969, 68.

33. N. Nishihkawa, K. Kamiija, A. Takabatake, H. Oshio, Y. Tomiie, and I. Nitta, Tetrahedron, 22, 3061 (1966).

34. S. J. Torrance, T. A. Geissman, and M. R. Chedekel, Phytochemistry, 8, 2381 (1969).

35. W. Herz and S. V. Bhat, J. Org. Chem., 37, 906 (1972).

36. S. M. Kupchan, V. H. Davies, T. Fujita, M. R. Cox, and R. F. Byran, J. Amer. Chem. Soc., 93, 4916 (1971).

37. S. M. Kupchan, M. Maruyama, R. J. Hemingway, J. C. Hemingway, S. Shibuya, T. Fujita, P. D. Cradwick, A. D. Hardy, and G. A. Sim, J. Amer. Chem. Soc., 93, 4914 (1971).

38. T. H. Porter, T. J. Mabry, H. Yoshioka, and N. H. Fischer, Phytochemistry, 9, 199 (1970).

39. H. Yoshioka, and T. J. Mabry, Tetrahedron, 25, 4767 (1969).

40. M. Sucky, V. Herout, and F. Sorm, Coll. Czech, Chem. Comm., 30, 2863 (1965).

41. N. H. Fischer, R. Wiley, Jr., H. N. Lin, K. Karimian, and S. M. Politz, Phytochemistry, in press.

42. T. C. Jarin and J. E. McClosky, Tetthedron Lett., 4525 (1969).

43. T. R. Govindachari, B. S. Joshi, and V. N. Kamat, Tetrahedron, 21, 1509 (1965).

44. M. F. Y'Homme, T. A. Geissman, H. Yoshioka, T. H. Porter, W. Renold, and T. J. Mabry, Tettrahedron Lett., 3161 (1969).

45. A. M. Shaligram, A. S. Rao, and S. C. Bhattacharyya, Tetrahedron, 18, 969 (1962).

46. R. W. Doskotch and F. S. El-Reraly, J. Org. Chem., 35, 1928 (1970).

47. A. S. Rao., A. S. Sadgopal, and S. C. Bhattacharyya, Tetrahedron, 13, 318 (1961).

48. W. E. von Doering and W. R. Roth, Tetrahedron, 18, 67 (1962).

49. R. Hoffmann and R. B. Woodward, J. Amer. Chem. Soc., 87, 4389 (1965).

50. H. Yoshioka, W. Renold, and T. J. Mabry, J.C.S. Chem. Commun., 1970, 148.

51. S. M. Kupchan, Y. Aynehchi, T. M. Cassady, A. T. McPhail, G. A. Sim, H. K. Schones, and A. L. Burlingame, J. Org. Chem., 34, 3867 (1969).

52. L. Dolej's and V. Herout, Coll. Czech. Chem. Comm., 27, 2654 (1962).

53. H. Yoshioka, T. J. Mabry, and A. Higo, J. Amer. Chem. Soc., 92, 923 (1970).

54. R. E. Alston, Evolutionary Biology I, (Meredith Publishing Co., New York, 1967), p. 266.

55. T. J. Mabry, R. E. Alston, and V. C. Runeckles, Editors, Recent Advances in Phytochemistry (Appleton-Century-Crofts, New York, 1968).

56. T. Swain, Editor, Comparative Phytochemistry (Academic Press, New York and London, 1966).

57. B. L. Turner, Pure Appl. Chem., 14, 189 (1967).

58. T. J. Mabry and D. R. DiFeo, Jr., Ecology, F. diCastri and H. Mooney, (Springer-Verlog, Heidelberg, and New York, 1973).

59. T. J. Mabry, Pure Appl. Chem., 34, 377 (1973).

60. R. H. Whittaker and P. P. Feeny, Science, 171, 757 (1971).

61. P. Tetenyi, Infraspecific Chemical Taxa of Medicinal Plants, (Chemical Publishing Inc., New York, 1970).

62. T. J. Mabry, Phytochemical Phylogeny, J. B. Harborne, Editor, (Academic Press Inc., London, 1970) pp. 269-300.

63. H. E. Miller, T. J. Mabry, B. L. Turner, and W. W. Payne, Amer. J. Bot., 55, 316 (1968).

64. J. L. Potter and T. J. Mabry, Phytochemistry, 8, 661 (1969).

65. W. W. Payne, J. Arnold Arboretum, 45, 401 (1964).

66. W. Renold, The Chemistry and Infraspecific Variation of Sesquiterpene Lactones in Ambrosia Confertiflora DC. (Compostitae), Dissertation, University of Texas-Austin (1970).

67. T. F. Stuessy, Rhodora, 74, 798 (1972).

68. T. F. Stuessy, Brittonia, 23, 177 (1971).

69. N. S. Bhacca, R. A. Wiley, and N. H. Fischer, J.C.S., Chem. Commun., 1973, 614.

70. R. T. Conley, Infrared Spectroscopy (Allyn and Bacon, Inc., Boston, 1966), pp. 140-142, and pp. 91-175.

71. N. S. Bhacca, F. W. Wehrli, and N. H. Fischer, J. Org. Chem., 38, 3618 (1973).

72. R. M. Silverstein, G. C. Bassler, and T. C. Morrill, Spectrometric Identification of Organic Compounds (3rd Ed.) (John Wiley & Sons, Inc., New York, 1974), pp. 5-40.

73. N. Wasada, T. Tsuchiya, E. Yoshii, and E. Watanabe, Tetrahedron, 23, 4623 (1967).

74. N. S. Bhacca and D. H. Williams, Applications of NMR Spectroscopy in Organic Chemistry, (Holden-Ray Inc., San Francisco, 1964) p. 108.

75. N. S. Bhacca and D. H. Williams, ibid., pp. 161-176.

76. S. Sternhell, Quart. Rev., 23, 236 (1969).

77. T. F. Stuessy, A Systematic Study of the Genus Melampodium (Compositae:Helintheae), Dissertation, University of Texas-Austin (1968).

78. C. Fishback, Masters Thesis, Louisiana State University (1974).

79. G. I. Poos, G. E. Arth, R. E. Beyler, and L. H. Sarett, J. Amer. Chem. Soc., 75, 425 (1953).

VITA

Donald Lee Perry was born in Oshkosh, Wisconsin on May 10, 1946. After moving to Neenah, Wisconsin at the age of five and attending the public schools, he graduated from Neenah High School in June, 1964. The following September he entered the University of Wisconsin-Fox Valley. In June, 1968, Mr. Perry graduated from the University of Wisconsin-Madison with a B.S. in Chemistry and was promptly drafted into the military. Upon completion of his military service, including a year in Vietnam as a medic, he entered graduate school at Louisiana State University in 1970. Presently, Mr. Perry is a National Science Foundation Predoctoral Fellow and a candidate for the degree of Doctor of Philosophy with a major in Organic Chemistry and a minor in Biochemistry.

EXAMINATION AND THESIS REPORT

Candidate: Donald Lee Perry

Major Field: Chemistry

Title of Thesis: The Chemistry and Biochemical Systematics of the Sesquiterpene Lactones in Melampodium (Compositae).

Approved:

Nicholas H. Fischer
Major Professor and Chairman

James D. Ingraham
Dean of the Graduate School

EXAMINING COMMITTEE:

E. S. Youngthorn

Steven T. Watkins

George R. Newkome

D. K. Carpenter

Date of Examination:

April 11, 1975